A STUDY OF LINKAGE AND TRANSLOCATIONS IN BARLEY

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ABSTRACT

The study of the inheritance and linkage of six morphological and chlorophyll-deficiency mutations, promising as sources of marker genes has yielded the following information:

1. The gene for "sterile 'brachytic'-like dwarf" (d_{ms}) is located approximately 0.6 crossover units from the gene conditioning light-green seedlings (lg) on linkage group I. The striping of the "cornstalk" mutant, which shows complex inheritance, is also associated with group I.
2. Genes for "yellow stripe" (ys) and "absent lower laterals" (als) are located on linkage group VI with the following gene order, als - uz - ys - a_n.
3. The gene for "mottled-3" (mt_3) is inherited independently of one or more marker gene pairs in each of six linkage groups, leaving the possibility that it may be in the new group VII.
4. Two genes for "long weak basal internode" are located: lwb, 1.5 crossover units from the gene conditioning glossy seedlings (gl_2) on group IV and lwb_2 on group III - VII, 20 crossover units from the naked caryopsis gene (n).
5. The gene order als - uz - ys - a_c - x_c - a_n - zb and the position of the centromere are suggested for linkage group VI on the evidence of crosses with the translocation C 1432.

The mutants studied are evaluated as to their value as marker genes.

The chromosomes involved in six translocations are identified and a seventh involves either d-e or d-f chromosomes. The d-e and d-f translocations are of particular value to the present available selection of barley translocations.

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INTRODUCTION

As the result of radiation work, a number of mutants in cultivated barley have been produced in recent years. Several of these, in addition to numerous spontaneous mutants, have proven of great value in elucidating the genetic linkages in barley. In addition to the linkage studies entailed in such work, numerous workers are at present employing newer techniques involving the use of translocations and karyotype analysis. These methods are presently making possible the correlation of barley linkage data with specific chromosomes.

Three main fields of study therefore exist, with full cooperation between them: the marker gene linkage studies in various countries and to some extent coordinated by Dr. D. W. Robertson and his associates in the United States; the karyotype studies headed by Swedish workers; and the translocation studies headed by Minnesota workers.

Considerable advances in our knowledge have recently been achieved by the use of these various techniques. Nevertheless, considerable gaps exist and require further extensive study. For example, the location of the centromeres in the linkage groups have yet, with one exception, to be determined and marker genes have not yet been associated with certain regions of the chromosomes. Moreover, the lack of a linkage group has been made apparent by the recent combination of two former linkage groups with one chromosome. There is also a need for additional translocation lines if efficient use is to be made of them for linkage studies.

The first part of this study was undertaken for the purpose of determining the inheritance and linkage relations of barley mutants that have been produced by radiations and various other treatments at the University of Alberta. The second part was undertaken with the hope of identifying useful barley translocations.

LITERATURE REVIEW

A. General

A number of comprehensive reviews of the literature on the genetics and cytology of barley have been made available. The most complete review of this wide subject was that of Smith (39) which listed over 900 articles in the bibliography. Burnham (4) reviewed the study of translocations in barley, and Burnham and Hagberg (7) gave an up-to-date account of the position of translocation studies in barley genetics. The summaries of Robertson (34) and Robertson et al. (35, 36, 37) correlated the available information of barley linkage studies. The recent institution of the "Barley News Letter" has supplied an up-to-date review of barley studies (8, 19, 32) from North America.

The common cultivated barley, Hordeum vulgare, has 7 pairs of chromosomes. Smith (39) stated there was a general agreement among barley cytologists that two of the pairs may be distinguished from the rest by the presence of satellites in root-tip cells. Identification and naming of the individual chromosome pairs has been based on the total relative lengths of the chromosomes, and on the ratio of the short arm to the long arm (15, 42). Other systems of karyotype descriptions have been made (Lewitsky, 1931; Oinuma, 1952) but these have not been adopted by leading barley cytologists in Sweden and the United States (7).

B. Mutation

For the purposes of this study, the definition of a gene as a unit of recombination has been adopted, as crossing-over and the standard

F_1 test for allelism are the only possible criterion that can be employed. In other instances, the gene as a unit of function or as a unit of mutation may be functionable. No methods are available to distinguish between "point" mutations and those caused by chromosomal aberrations in barley, and therefore the word "mutation" in this thesis will not be used in the refined sense of "gene-mutation" commonly used by geneticists. The radiation- and chemically-induced mutants in barley, in the absence of definite criteria such as the back mutations used in Neurospora, may may not be conclusively presumed to be gene mutations, but may be the result of chromosomal aberrations. The term "mutants," as used here, refers therefore to relatively rare heritable variants, either chromosomal or genic.

Many mutations in other species, previously considered to be point mutations, have been shown to be associated with chromosomal aberrations and therefore considered to be the result of these aberrations. For example, Dollinger (10) has shown that the majority of mutations, induced at three different loci in maize, were associated with detectable chromosomal aberrations.

Smith (40), however, presented the conservative view that whether true "point" mutations, unaccompanied by breaks in and rearrangements of the chromosome, can be produced depends upon the nature of the longitudinal cohesive forces holding the chromosome intact and of the functioning of the ultimate units of heredity.

In a discussion of the relation of mutation to "positive effect," McClintock (29) explained the possibility that the change in expression of a character may be associated with either the interaction of other

closely associated loci or the proximity of heterochromatin to the particular loci, caused by changes of position on the chromosome.

Another type of mutation, "block" mutation, was stated by von Wettstein (45) to have occurred in barley. A "block" mutation refers to the simultaneous mutation of a group of very closely linked but distinct genes.

Induced mutations in barley have been reported in articles too numerous to mention here. However, excellent summaries were presented by Gustafsson (12, 13), Nybom (31) and Smith (39).

Chlorophyll-deficient mutations have been the most abundant type of mutation reported in barley (39), with almost every conceivable chlorophyll variation observed. The approximate order of frequencies were white, yellow-green, virescent, yellow, transversely zoned and longitudinally striped. Gustafsson (13) stated that only one in about twenty-five chlorophyll mutations are viable in the homozygous state. Smith (39) reported that fluctuations in the expression of chlorophyll-deficient types are frequently observed, mainly due to environmental effects. He also mentioned that, almost with exception, the chlorophyll mutations studied have been reported to be simple recessives.

In recent studies (11, 27, 32), it appeared that dwarf forms are about the most abundant morphological mutation obtained from irradiation studies of barley.

Burnham and Hagberg (7) have outlined a method of rating barley gene pairs on the basis of their usefulness as marker genes as follows:

"1. Endosperm characters - classified on F_1 plants.
2. Seedling characters with viability and vigour equal to that of normal plants.
3. Seedling characters, not lethal but lower in viability.
4. Lethal seedling characters.
5. Adult plant characters that are as vigorous as normals.
6. Same as 5, but lower fertility (includes male-steriles).
7. Adult plant characters with lowered viability.
8. Relatively unusable characters due to poor expression or complex inheritance."

C. Genetics and Cytology of Barley

Robertson et al. (35), upon summarizing and standardizing the barley linkage data, listed seven groups of gene pairs which they designated by the Roman numerals I - VII. Each of these seven groups was considered to correspond to one of the seven pairs of chromosomes present in cultivated barley. However, Kramer, Veyl and Hanson (26) subsequently associated linkage groups III and VII with a single chromosome, employing chromosomal interchanges in conjunction with linkage-group marker genes. Burnham (5) later confirmed this finding and Haus (19) has established linkage associations between the gene markers A_{c2} a_{c2} on group III and Yc yc on group VII. Takahashi (personal communication) independently found associations between these two genes as well as associations between them and other genes

on these linkage groups. The most recent reports listed these two as the combined linkage group III - VII (7, 14, 34).

Table I is adapted from Table 3 of Burnham and Hagberg (7) with alterations as suggested from recent articles (19, 33) and communications. This table shows the linkage groups of barley as knowledge of them exists at present with the generally accepted relative positions of the genes.

Smith (39) cites several references dealing with genetic studies of barley diseases. "Barley Stripe," which is caused by Helminthosporium gramineum, has been found to vary in its expression according to several factors, especially environment. Arny (3) found that the number of genes involved in the "stripe" reaction varied from 1 to 3 or possibly more. He found no linkage associations with marker genes on five of the presently recognized linkage groups.

D. Barley Translocations

Two naturally occurring translocations were studied in barley by Smith (38) in 1941. Since that time, numerous translocations have been induced and studied in greater detail (cf. Burnham, 4, for summary).

Burnham, White and Livers (6) described a method of distinguishing the seven pairs of chromosomes, based on intercrossing chromosomal interchanges. They established a tester set of translocation lines, for use in identifying the chromosomes involved in other interchanges, for linkage tests with various linkage group markers,

Table I. Linkage groups in barley with recombination values reported between certain loci included. (Adapted from Table 3 of Burnham and Hagberg, 7)

Linkage group		Not placed but useful factors
I*	tr 39 li 9 Pc V Pr 25 rin g re_2 h e f y 5 lg or	ms_2
II	B 13 trd 16 a_t Bt	
	a_t 12 ml_d 16 ml_p	
III-VII**	n 10 Bl_2 9 Rs a_{c2} 28 yc 30 wx 1.5 fc 10 br 13 t	ms
IV	I 14 K lg_4 15 z lg_3 gl 10 gl_2 ml_g 26 Bl	
V	lb 16 R 25 l_3 23 S fs	
VI	uz 11 a_c x_c 5 lc a_n 16 x_s	zb, Bt ($Bt\text{-}a_n$ = 0.86%)
VII***		ec, o

* Woodward (46) suggested the following gene order for linkage group I tr - re_2 - V - Pr - e - li, with Pc at the same locus as re_2.

** Haus (19) obtained a recombination value of A_{c2} a_{c2} - Yc yc = 28 while Takahashi (personal communication) obtained smaller values.

*** Ramage and Suneson (33) associated a gene (ec) for earliness with the g chromosome which previously had no markers located on it. Evidence indicates that orange lemma (o) is not associated with group V as previously reported, but is associated with the new group VII.

Note: Characters normally referred to by their dominant allele are capitalized.

and for locating markers for the new linkage group rendered probable by the union of groups III and VII. The seven pairs of chromosomes as distinguished by chromosomal interchanges were given the temporary designations a to g until a comprehensive system of nomenclature could be established.

Later, Burnham and Hagberg (7) proposed a new Arabic system of nomenclature which would correlate the karyotype designations of Tjio and Levan (42), the linkage groups as summarized by Robertson et al. (35, 36, 37) and the temporary designations of Burnham, White and Livers (6). The partial coordination of this Arabic system with those mentioned is shown in Table II, abbreviated from Table 2 of Burnham and Hagberg (7).

Table II. A summary of the correspondence of various systems of designating chromosomes and linkage groups in barley

Reference	Chromosomes							Based on
Tjio and Levan (1950)	I	II	III	IV	V	VI	VII	root-tip cytology
Proposed: Burnham & Hagberg (1956)	1	2	3	4	5	6	7	
Burnham et al. (1954)	f	b	c	e	a	g	d	interchange intercross
Robertson et al. (1955)	I	III + VII		VI	IV	II	-	V? linkage data

? Linkage group V has not been definitely associated with the d chromosome

In a still more recent publication, Hagberg (14) has insisted that the proposed chromosomes 1, 2 and 3 (based on karyotype analysis) cannot yet be definitely associated with the specific chromosomes b, f

or c (which have been associated with the linkage groups). However, he has interchanged the b and f chromosomes in relation to the proposed 1 and 2 chromosomes.

A new linkage group VII has been started with the association of a gene for earliness with the g chromosome. Ramage and Suneson (33) obtained this association by crossing the line carrying this gene with a number of selected translocation lines and classifying the resulting F_2 progenies for partial sterility. Apparently the gene for orange lemma has also been associated with the g chromosome (personal communication).

Burnham (4) stated that in translocation heterozygotes of species such as barley, which have a high proportion of "directed" segregation of chromosomes of the translocation complex, crossing-over is reduced in the interstitial segment. Hanson and Kramer (17) proposed that this reduction of crossing-over involving interchanges with break points at different loci in a linkage group may give a clue as to the position of the centromere. Hanson (16) illustrated such an instance with linkage group IV of barley.

E. Linkage Calculations

The basic assumption for the calculation of linkage intensities is that the percentage of recombination of any two linked genes remains fairly constant, and thus the distance between any two linked genes is a function of the number of crossovers that occur between them. This, however, does not always hold true, especially for genes located farther apart, as corrections must be made for multiple

crossovers and for interference. These corrections cannot be made for F_2 data and it must be assumed that crossing-over is equal in both sexes for such data. Other factors which are less readily accounted for, such as chromosomal aberrations and the degree of heterogeneity of the crossing lines, may result in variations of crossover values. Therefore, the values obtained in any study can only be assumed approximate and subject to deviations.

As discussed by Immer and Henderson (21), the two methods of greatest utility for the calculation of linkage intensities from F_2 and F_3 data are the Product Method and the Method of Maximum Likelihood.

Tables for linkage calculations by the Product Method are provided by Immer (20) and Immer and Henderson (21). Kramer and Burnham (25) have derived the Maximum Likelihood formulae for the calculation of linkage and have adopted Fisher's Scoring Method for recombining F_2 and F_3 data. Allard (1) has provided formulae and tables for the calculation of linkage intensities with various other types of ratios besides the dihybrid, based on the Method of Maximum Likelihood. Allard's tables may also be used for the advance planning of population sizes required for specific linkage investigations. He also discussed the efficiency of growing F_3 progenies from F_2's where linkage indications have been obtained.

A third method for the calculation of linkage has been proposed by Murty (30), called the Minimum Discrepancy Method. He also included formulae for calculations where segregation ratios other than the 9:3:3:1 ratio are involved.

Mather (28) has given a fairly complete review of methods of treating linkage measurements. He has also described the detection of linkage by the separation of the total X^2 value into three components, the third component being that of interaction (or linkage).

Kramer and Burnham (25) have briefly reviewed the formulae to use when calculating linkage intensities from various sources of F_2 and F_3 data:

(a) For F_2 data alone use the Product Method (20, 21).

(b) For F_3 data from singly dominant F_2 phenotypes, use the Product Method (21).

(c) For F_3 data from doubly dominant F_2 phenotypes, use the Maximum Likelihood Method (25).

(d) For a combined p value which best fits all available data, use Fisher's Scoring Method (25).

The more recent article of Allard (1) has provided more adequate tables and formulae to facilitate the use of the Maximum Likelihood Method and should be followed except where it is possible to use the Product Method for linkage determinations.

For the calculation of linkage intensities from data classified for partial sterility due to chromosomal interchanges, Joachim (22) provided tables and formulae for the Product Method. Later, Hanson and Kramer (18) derived the Maximum Likelihood formulae for the calculation of linkage intensities and adapted Fisher's Scoring Method for the recombining of F_2 and F_3 data where random sterility is involved. Kramer (24) has summarized the formulae for the Method of Maximum Likelihood where linkages are determined with the translocation point.

PART I: LINKAGE STUDIES

MATERIALS AND METHODS

The materials used in this study were obtained from an extensive collection of barley genetic stocks at the University of Alberta originated in 1954 by Dr. G.W.R. Walker. This collection contained, in addition to genes already located and useful as marker genes, a group of unassigned mutants produced in Canada.

The mutant lines studied were selected from this latter group, produced in Montcalm with several types of ionizing irradiations by Lawrence (27) and in Gateway with chemical treatments by Kerber (23). A further mutant in Montcalm (Acc. #263) of unknown origin, from the University of Saskatchewan was also included. These principal mutants are listed in Table III, the Accession numbers referring to Dr. Walker's collection.

Table III. Mutant lines studied for mode of inheritance and linkage associations

U. of A. Acc. No(s)	Mutant name at source	Proposed symbol	Treatment at mutant origin
332	Sterile "brachytic"-like dwarf	dms	Acetone trt. Gateway seedlings
262	Cornstalk	-	Unknown - Montcalm
289	Long weak basal internode (Crooked neck)*	lwb)	Various irradiations o
291-293	Long weak basal internode (Crooked neck)	lwb_2)	Montcalm with x-rays,
290	Long basal internode (Crooked neck)	lb_2)	Co^{60} (low and high trt.
326	Yellow striped	ys	D.D.T. trt. Gateway sdl
281	Absent lower laterals	als	Co^{60} low trt. of Montcal
280	Mottled-3	mt_3	radium-beryllium trt. of Montcalm

* These lines were originally designated "Crooked neck" by Lawrence (27); however, "long basal internode" is proposed as it gives a more precise description of the mutant character expression.

A number of adequately spaced and positioned genes were selected as markers for the various linkage groups, with the addition of a few quite extensively tested but unlocated genes. Where linkage associations were obtained with a linkage group, other useful markers in that group were also crossed to the mutant. Table IV lists the various marker genes used, their associated linkage group and the accession number of the marker stock.

The general method followed involved making preliminary crosses with three tester lines: Acc. #* 8 (Nigrinudum) or Acc. #82 (Nigrillaxum); Acc. #11 (Brachytic 119); and Acc. #9 (Colsess V Chlorina) during the summer of 1956 and winter of 1956-57. These preliminary crosses were grown in the greenhouse during the winter and the F_2 seed planted in the field in the spring of 1957. These F_2 populations were classified for linkage associations with at least one segregating marker gene in each of the six well-established linkage groups (with the exception of group VI where the parent plant of Acc. #8 was not segregating for white seedlings $\underline{A_n}$ $\underline{a_n}$). Where the $\underline{A_n}$ $\underline{a_n}$ gene was not segregating in these crosses with Acc. #8, the crosses were repeated if required.

Where linkage associations were observed from preliminary crosses, as with Acc. Nos. 263, 332 and 289, further crosses with markers in the associated linkage group were attempted in the summer and fall (in greenhouse) of 1957. If no linkage associations were observed from the segregations of preliminary crosses, further crosses

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* The abbreviation "Acc. #" will be used to designate Accession number.

Table IV. List of marker gene pairs used for location of mutants

Linkage group	Gene symbol	Marker character pair	Acc. no(s) of marker lines
I	V v	non 6-row vs. 6-row	8, 13, 82, 147, 152
	E e	normal vs. long-awned glume	147
	Log log	normal vs. long-awned glume	152
	Tr tr	normal vs. triple-awned lemma	147, 152
	Lg lg	green vs. light-green seedlings	123
	Pr pr	purple vs. nonpurple stem	37
	Li li	normal vs. liguleless	366
	Ms_2 ms_2	normal vs. male-sterile-2	49
II	B b	black vs. white lemma and pericarp	8, 82
	Trd trd	normal vs. third outer glume	13
	A_t a_t	normal vs. white seedlings	5
III-VII	N n	covered vs. naked caryopsis	8, 11, 82, 107
	Rs rs	red stem vs. green stem	377
	Yc yc	normal vs. virescent seedlings	376
	Br br	normal vs. brachytic	11, 107
	Fc fc	normal vs. chlorina seedlings	9
	Ms ms	normal vs. male-sterile	47
IV	K k	hooded vs. awned	9, 82, 107
	Z z	normal vs. zoned leaf	43
	Gl gl	normal vs. glossy seedlings	118
	Gl_2 gl_2	normal vs. glossy seedlings	371
	Lg_2 lg_2	green vs. light-green seedlings	128
	Lg_3 lg_3	green vs. light-green seedlings	124
	Lg_4 lg_4	green vs. light-green seedlings	383
	I^h i	fertile intermedium vs. non-intermedium	8
V	R r	rough vs. smooth awns	8, 11
	S s	long vs. short-haired rachilla	8, 9
	Lb lb	normal vs. long basal spike internode	385
VI	Uz uz	normal vs. "uzu"	359, 375
	A_n a_n	normal vs. white seedlings	8
	St st	normal vs. streaked-163	386
Unlocated markers	Gp gp	normal vs. grandpa	259
	Rb rb	normal vs. ribbon grass	388
	Fs_2 fs_2	normal vs. fragile stem-2	359
	*	normal vs. Gateway male-sterile-1	336
	*	normal vs. Gateway male-sterile-2	337

* These unlocated male-sterile factors have not yet been assigned a symbol.

were made involving at least one or two more well separated genes on each linkage group.

The F_2's of these further crosses made in 1957 were sown in the field in the spring of 1958. Where seedling classification of F_3's was possible from F_2's indicating linkage associations, the F_3 seed was harvested in early August, 1958. The freshly harvested heads were dried in an oven at 32 - 33° C. for 3 days, then arranged in foil cake plates and moistened with a light covering of Vermiculite. The moistened heads were placed in a cold chamber at 12° C. for 3 days to induce germination. The heads, each representing an F_3 progeny, were planted in the greenhouse and the seedlings were classified 10 days to two weeks later. An attempt to grow F_2 progenies, where both characters were classifiable in the seedling stage, in foil cake plates and covered with Vermiculite met with varying success.

Spikes of the F_1 generation of the crosses were collected for cytological study of the microsporocytes. They were fixed in Carnoy's 6:3:1 solution at room temperature for 2 days, then stored under refrigeration until examined.

A number of barley translocation lines produced in Minnesota and Sweden were supplied by Professor Shebeski of the University of Manitoba. From the translocations originating at Minnesota in the variety Mars, a set of five testers (6) were selected (Table V) for Part II of this study. They were planted in the greenhouse in September, 1957, along with the mutant lines being studied in Part I. Since no linkage

Table V. Tester set of chromosomal interchanges from the variety Mars

Designation at source	Chromosomes interchanged	Linkage groups tested		Mutant lines crossed to
C 1358	b - d	III - VII	V?	281, 326
C 1483	b - g	III - VII	new VII	281, 326
C 1405	c - d	VI	V?	281, 326
C 1432	c - e	VI	IV	280, 281, 326
C 1420	e - f	IV	I	280, 281, 326

indications with Acc. lines 326, 280 and 281 had been obtained up to that time, these lines were crossed with the translocation tester set to check for linkage associations. The general procedure and analysis were carried out according to the outline of Kramer, Veyl and Hanson (26).

Linkage relationships were determined on the interaction of characters in the F_2 populations. Where possible, F_3's of F_2 populations were classified for increased accuracy of the linkage estimates.

Fisher's breakdown of the X^2 (28) was employed for the detection of linkage from F_2 populations. Recombination percentages were calculated by two methods, based on the outline of Kramer and Burnham (25):

(a) The Product Method, with the aid of tables of Immer and Henderson (21). The tables of Joachim (22) were used for the calculation of linkage from F_2 populations involving chromosomal interchanges.

(b) The Maximum Likelihood method, with the aid of formulae and tables of Allard (1) and Kramer and Burnham (25).

F_2 populations of 450 seeds were planted where possible, with the aim of obtaining at least 400 F_2 plants. Certain crosses failed to yield the desired number of F_2 seeds while F_2 populations were reduced by cutworms in the springs of 1957 and 1958. The average reduction of F_2 population size was approximately 20% in 1958.

The writer feels that until definite associations of the new proposed Arabic system of nomenclature have been established, use of this system would possibly lead to confusion and has therefore used the linkage group designations and the temporary designations of Burnham *et al.* (6) for the chromosomes.

To avoid awkwardness arising from the continual use of long mutant names, the writer has made use of symbols (such as "$\underline{d_{ms}}$ $\underline{d_{ms}}$") throughout the Results and Discussion.

RESULTS AND DISCUSSION

1. The association of two mutants with linkage group I

(a) The first mutant, "sterile 'brachytic'-like dwarf" (d_{ms}) appears to be conditioned by a single recessive gene, inducing dwarf habit and sterility (Fig. 1). The dwarf habit is very similar to that caused by the gene br located on linkage group III-VII (35), except that the d_{ms} d_{ms} plant is a bit shorter. The sterility is caused by the failure of pollen formation; the dwarfs will set seed when used as the female parent in crosses. Some of the heads show a tendency towards compound florets by the appearance of floral fasciation (Fig. 1).

Results

In an attempt at determining whether the two main characters of the mutant (dwarf habit and male-sterility) are conditioned by the same gene or by two closely linked genes, a number of male-sterile lines were crossed to the "sterile 'brachytic'-like dwarf" line. These consisted of:

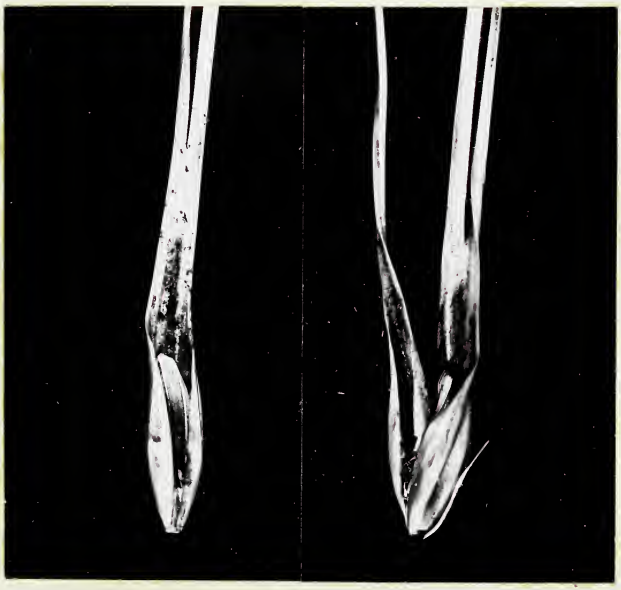
a) ms ms (Acc. #47)

b) ms_2 ms_2 (Acc. #49)

c) and d) two as yet unregistered male-steriles isolated in Gateway by Kerber (23).

The F_1's were all completely fertile and progenies of the heterozygous parent in each cross were grown to establish the presence of both recessive genes in each of the crosses. Therefore, if there are two components of the mutant gene, at least the male-sterile component is not allelic with any of the four known male-sterility genes.

(a)



(b)

Fig. 1. "Sterile 'brachytic'-like dwarf" (d_{ms}).
(a) Growth habit. (b) Sterile florets showing fasciation and multiple floret tendencies.

Intercrosses of the four male-sterile tester lines indicated that none were allelic.

Table VI shows the F_2 interrelations of $\underline{d_{ms}}\ \underline{d_{ms}}$ with various marker genes. Classification of F_2 plants from the preliminary cross with Acc. #8 gave linkage associations with 2-row vs. 6-row ($\underline{V}\ \underline{v}$) on linkage group I. Further crosses with other marker genes located on linkage group I were then made. From a total of 2115 F_2 plants classified in Table VI, 519 were $\underline{d_{ms}}\ \underline{d_{ms}}$ giving a X^2 value of 0.325 for the goodness-of-fit to the expected 3:1 ratio of $\underline{D_{ms}}$- $\underline{d_{ms}}\ \underline{d_{ms}}$. The expressivity of triple awned lemma ($\underline{tr}\ \underline{tr}$) was very weak, often limited to one floret of a spike. A poor fit to the expected 3:1 ratio of $\underline{Tr}$- to $\underline{tr}\ \underline{tr}$ was obtained, and it is believed that this is an effect of reduced penetrance.

The results of Table VI indicate that the segregation for $\underline{D_{ms}}\ \underline{d_{ms}}$ is independent of the following:

black vs. white pericarp ($\underline{B}\ \underline{b}$) in linkage group II

normal vs. chlorina seedlings ($\underline{Fc}\ \underline{fc}$) in group III - VII

hooded vs. awned ($\underline{K}\ \underline{k}$) in group IV

rough vs. smooth awns ($\underline{R}\ \underline{r}$) in group V

normal vs. liguleless ($\underline{Li}\ \underline{li}$)

and normal vs. triple-awned lemma ($\underline{Tr}\ \underline{tr}$) in group I.

Significant deviations of the X^2_L value indicate that the following marker genes for linkage group I are not inherited independently of $\underline{D_{ms}}\ \underline{d_{ms}}$:

non 6-rowed vs. 6-rowed ($\underline{V}\ \underline{v}$)

normal vs. long-awned glume ($\underline{E}\ \underline{e}$)

normal vs. long-awned glume ($\underline{Log}\ \underline{log}$)

Table VI. F_2 segregations of marker character pairs with "sterile 'brachytic'-like dwarf" (d_{ms}) Acc. #332

Linkage group	Phase at cross	Marker Acc. No(s)	Genotypes tested		F_2 phenotypic frequencies					X_L^2 [7]	Level of probability
			X x	Y y	X Y	X y	x Y	x y	Total		
I	Coup.	8, 82, 147, 152	V v	D_{ms} d_{ms}	954	169	176	178	1477	174.035	very small
	Rep.	147	E e	"	195	73	78	0	346	21.373	.01.
	Rep.	152	Log log*	"	310	141	169	0	620	68.888	very small
	Rep.	123	Lg lg	"	202	69	86	0	357	21.528	.01
	Coup.	37	Pr pr**	"	128	21	24	19	192	15.565	.01
	Rep.	366	Li li	"	126	43	39	12	220	0.073	.7 - .8
	Rep.	147, 152	Tr tr	"	618	170	134	44	966	1.197	.2 - .3
II	Coup.	8	B b	"	60	21	17	10	108	1.333	.2 - .3
III-VII	Rep.	9	Fc fc	"	170	71	78	21	340	2.531	.1 - .2
IV	Coup.	9, 82	K k	"	203	65	74	31	373	1.259	.2 - .3
V	Coup.	8	R r	"	52	28	25	3	108	6.584/	.01 - .02

7 X_L^2 is X^2 value for linkage as described by Mather (28). The 5% level of significance is 3.841 with 1 D.F.

* Robertson et al. (37) propose that, as Log log and W w are similar in expression of character to E e, the symbol E e be used to designate all such characters.

** The name of Acc. #37 at its source was "71-Pr Pr-13," however, Robertson et al. (37) propose the symbol Re re for purple vs. non-purple grain while they use Pr pr for purple vs. non-purple stem. The character expression classified as the marker character from Acc. #37 was purple vs. white grain. Takahashi et al. (41) use Pr pr for purple vs. white sheath and kernel.rows.

/ This value must be due to a chance deviation as cross was made in coupling and the deviation is in the opposite direction to that expected.

normal vs. light-green seedlings (Lg lg)

and purple vs. nonpurple stem (grain) (Pr pr).

The recombination values between different gene pairs on linkage group I calculated from the F_2 segregations are:

$D_{ms}\ d_{ms}$ - V v = 28.34 ± 1.42%

$D_{ms}\ d_{ms}$ - E e, Log log, Lg lg = no recombinants

$D_{ms}\ d_{ms}$ - Pr pr (Re re) = 29.28% ± 4.13%

$D_{ms}\ d_{ms}$ - Li li = 48.52 ± 5.14%

V v - E e = 33.32 ± 4.71%

V v - Log log = 24.37 ± 3.74%

V v - Tr tr = 49.54% $\quad X^2_L = 0.056$

E e - Tr tr = 55% $\quad X^2_L = 0.289$

Log log - Tr tr = 51.69% $\quad X^2_L = 0.734$

These F_2 results indicate that $d_{ms}\ d_{ms}$ is to the right of V v and in the region of the E e and Lg lg loci according to the accepted gene order as given in Table I. (Note: A diagram will be shown later in the discussion to indicate the various gene positions).

The value ($D_{ms}\ d_{ms}$ to V v) was obtained by combining the data from six different crosses. Table VII lists the six sets of data with the phenotypic frequencies and recombination values obtained.

To recombine the data of Table VII, Fisher's scoring method as described by Allard (1) was used with the aid of Allard's tables.

Table VII. F_2 data of various crosses showing the interrelations of the V v and D_{ms} d_{ms} gene pairs

Data set no.	Phase of cross	Marker Acc. #	F_2 phenotypic frequencies				Total	Recombination value
			V D_{ms}	V d_{ms}	v D_{ms}	v d_{ms}		
1	Coupling	8	94	21	7	16	138	22.10%
2	Coupling	82	236	55	41	41	373	31.05%
3	Coupling	147♂	89	12	22	20	143	27.03%
4	Coupling	147♀	127	20	35	21	203	33.28%
5	Coupling	152	208	30	34	36	308	25.16%
6	Coupling	153	200	31	37	44	312	24.89%

Table VIII shows the calculations where a first appropriation for the p value of .28 was chosen. The data allow a calculation of a X^2 value which indicates the heterogeneity of the data. If a significantly high X^2 value is obtained, the data should not be combined, since different loci are probably involved in the various sets of data.

Table VIII. An estimation of the combined recombination value of V v - D_{ms} d_{ms} data and a test of heterogeneity by Fisher's Scoring Method

Data set no.	p = .28		
	Score	Information	X^2
1	-14.4719	463.542	0.4518
2	38.2115	1252.907	1.1654
3	- 4.7839	480.337	0.0476
4	33.5010	681.877	1.6459
5	- 2.3705	1034.572	0.0054
6	-33.2583	1048.008	1.0554
Total	+16.8278	4961.243	4.3715

Correction = $\frac{16.8278}{4961.243}$ = .0034 $\therefore$ p = .2834 S. E. = $\frac{1}{4961.243}$ = ±.0142

The X^2 value at the 5% level of significance with 5 degrees of freedom is 11.070. Therefore the X^2 value of 4.3715 is not a significant deviation and the sets of data may be combined to obtain a best-fit value of 28.34 ± 1.42%.

F_3 seed from the singly dominant F_2 phenotypes $\underline{D_{ms}}$- $\underline{e}$ $\underline{e}$, (harvested, dried and germinated under cold temperatures as outlined under Materials and Methods) germinated poorly. However, 57 F_3 progenies contained 10 or more seedlings each and of these, 5 segregated for dwarfs. (Ten plants per progeny give 95% accuracy for distinguishing between heterozygous and homozygous F_2's). F_3 seed from singly dominant F_2 phenotypes $\underline{D_{ms}}$- $\underline{lg}$ $\underline{lg}$, similarly harvested and germinated, gave good germination with over 16 seedlings in each F_3 progeny. From 85 such F_3 progenies, only one segregated for dwarfs.

Using Table 3 of Immer and Henderson (21), the F_3 recombination values were:

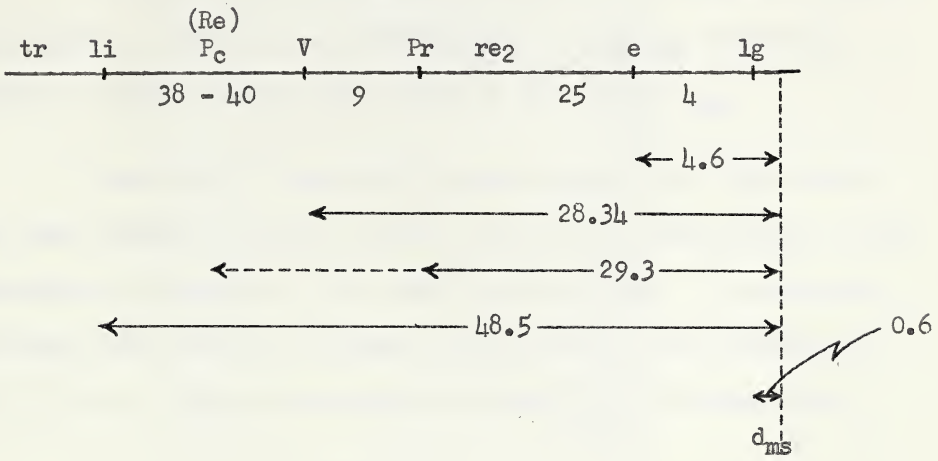
$\underline{D_{ms}}$ $\underline{d_{ms}}$ - $\underline{E}$ $\underline{e}$ = 4.59 ± 2.05%

$\underline{D_{ms}}$ $\underline{d_{ms}}$ - $\underline{Lg}$ $\underline{lg}$ = 0.59 ± 0.45%

Discussion

The accepted gene order for linkage group I is given in Table I on the basis of summaries of Robertson *et al.* (35, 36, 37) who are the authorized coordinators of barley linkage data. The following diagram gives the relation of the linkage values obtained

from this study, in relation to the accepted gene order.



Robertson et al. (37) and Takahashi et al. (41) placed the genes Tr tr and Li li at the far left of linkage group I, however, Woodward (46) recently suggested that Li li should be at the far right of the group. The results of this study support the former (37, 41) articles. Robertson et al. (37) and Woodward (46) placed Pr pr to the right of V v while Takahashi et al. (41) placed Pr pr to the left of V v. The results of this study are not conclusive with respect to the character classified or the position for purple vs. nonpurple grain.

Immer and Henderson (21) obtained the following values for marker genes in group I:

V v - E e = 28. ± 1.2%, V v - Lg lg = 31. ± 2.5%

and E e - Lg lg = 4. ± 1.2%.

These values give the accepted gene order of v - e - lg. Therefore the gene D_{ms} d_{ms} fits the accepted gene order if it is positioned very close to Lg lg, possibly on either side.

A further check on the gene order can be obtained by growing F_3 progenies of the crosses involving E e and Log log which will provide a 3-point linkage test with V v, E e and D_{ms} d_{ms}.

The review of Smith (39) listed several dwarf forms that have been studied in barley; however, none fit the description of the "male-sterile 'brachytic'-like dwarf" in this study. In particular, the dwarf gene located by Woodward (46) on group I at a distance of 31.5 ± 1.9 c.o. units from long-awned glume (E e) is self-fertile.

Concerning the usefulness of d_{ms} d_{ms} as a marker gene, it should be given a rating of "6" according to the system of Burnham and Hagberg (7).

(b) Inheritance studies with "cornstalk" Acc. #263

The material for this study was obtained from the University of Saskatchewan, where it obtained the designation of "cornstalk." As the name indicates, the mutant produces only a few large, thick tillers. The line also contains a broad pale striping which appears on the leaves 3 - 5 weeks after emergence. Figure 2 shows the extreme expression of the "cornstalk" mutant, with only one to two tillers maturing, from a basal rosette of tillers with striped leaves. Although the parent line is true-breeding for the striping, all the plants do not exhibit a reduced number of tillers. The heads, except for an occasional blight of the top, are well-filled and the seeds are viable.

Fig. 2. "cornstalk," Montcalm mutant

Results

In the summer of 1956, selections were made from the parental material of cornstalk to try to obtain a line which was uniform for the reduced tillering, but did not prove successful. The F_1 plants, from crosses made with "cornstalk" as the female parent in 1956, were of a normal green appearance, under both field and greenhouse conditions. It was observed that the striping of plants from the parent "cornstalk" line was absent or difficult to detect under greenhouse conditions.

Three other lines in the collection of barley genetic stocks at the University of Alberta, grown in the summer of 1957, were observed to be similar in appearance to "cornstalk." Therefore Acc. Nos. 257 and 317 (designated as "mid-season broad stripe" and

"striped" respectively) were crossed with Acc. #263 and the F_1 plants were grown in the summer of 1958. All the plants developed striping, and since this character behaves as a recessive in other crosses, this indicated that the striping was conditioned by identical or allelic genes in these three lines.

In the spring of 1956, 150 F_2 seeds from a cross of "cornstalk" with Brachytic 119 (Acc. #11) (made by Dr. Walker) were planted. This progeny was observed occasionally throughout the growing season, but none of the plants exhibited either the striping or the reduced number of tillers.

The next step was to investigate the cause of the striping, which was originally assumed to be physiological due to a gene mutation. Striping, however, has been associated in some cases with fungal, bacterial and viral infections.

The striping of "cornstalk" appears to be somewhat similar to the Barley Stripe symptoms (caused by the fungus Helminthosporium gramineum) which have been described by Dickson (9). In the spring of 1958, two lots of parental seed of "cornstalk" were sown, one pretreated with a mercurial fungicide, Ceresan, and the other lot untreated. Both plots showed striping on all plants, indicating that the fungus H. gramineum was not the cause of striping. These results also indicated that the stripe is not Bacterial Blight (caused by Xanthomonas translucens). In this disease the bacteria are carried over on or near the surface of the seed, and, therefore, infection should have been controlled by the mercurial dust treatment.

The stripes on the "cornstalk" plants are quite broad and pale yellow in color while the striping of Barley Stripe darkens along the edges and conidia are produced. No conidia were found on the stripes of this mutant. The stripes of Bacterial Blight are associated with the main veins of the leaf and bacterial exudate is often present at the base of the leaves where the stripes originate. These conditions were not found in the striping of the "cornstalk" mutant.

Two main possibilities remain as an explanation of the cause of the striping:

a) a physiological gene-effect

b) a virus infection, similar to False Stripe.

The absence of secondary infections of neighboring progenies during the summer and the lack of striping on the F_1 plants indicate that the striping may be due to physiological effects rather than a virus infection.

In the spring of 1957, a "date of seeding" experiment was planned to check for environmental influences on the expression of the striping. F_2 plots of the cross with Acc. #8 (Nigrinudum) and a row of the parent line Acc. #263 were sown at three different dates, the first date being as early as weather and field conditions would permit. No differences could be detected in either the F_2 plots or the parent plots in relation to different dates of seeding. All plants in each of the parent-line plots were striped and the majority of plants in each showed reduced tillering with large stems. The segregation of non-striped to striped F_2's was as follows:

Date of seeding	Phenotypes: Non-striped	Striped	X^2	D.F.
May 10	137	30	4.409	1
May 22	132	30	3.630	1
June 1	210	52	3.710	1
		Total	11.749	3
Pooled	479	112	11.534	1
Homogeneity			0.215	2

To check for the environmental effect due to different dates of seeding, a test of homogeneity was applied on the basis of an expected 3:1 ratio where striping was caused by a single recessive gene. As all three plantings were from the same F_2 population, the test for homogeneity shows that environmental differences had no significant effect on the striping; however, the deviation from the expected 3:1 ratio was highly significant (11.534).

Other F_2 populations of "cornstalk" crossed with Acc. Nos. 11 and 107 (Brachytic lines), sown also on June 1, 1957, showed a marked reduction in the proportion of striped plants. A X^2 test for homogeneity

Marker line	Non-striped	:	Striped
Acc. #11 (Brachytic 119)	157	:	9
Acc. #107 (Brachytic L50-243)	166	:	8
Total	323		17

in these two populations gave a value of 1.031 with an approximate 0.3 level of probability (1 D.F.).

The drastic differences in the proportion of striped plants between the two groups of crosses indicate that the expression of striping in the F_2 populations is strongly influenced by the marker parents of the cross.

From the results of the F_2 population grown in the summer of 1956, where no striped plants were observed, and the large F_2 populations grown in the summer of 1958, it would appear that environment may still have some effect on the expression of the striping even though this effect was not significant in 1957 field progenies.

The striping in 1958 field plots was very poor as only 3 to 5.5% of the plants were striped. These F_2 progenies were from crosses with other marker genes on linkage group I. The percentage of striping was considered too small to obtain a significant test for linkage and therefore only the segregations of the striped plants were observed, as shown in Table IX.

Table IX. F_2 segregations of linkage group I marker genes in striped mutants of "cornstalk"

Marker Acc. #	Marker gene	Phase of cross	Striped phenotypes	X^2
147	V v	Coup.	7 V- : 7 v v	4.67
	E e	Rep.	13 E- : 1 e e	2.28
152	V v	Coup.	5 V- : 6 v v	2.45
	Log log	Rep.	10 Log- : 1 log log	1.49
123	Li li	Rep.	6 Li- : 2 li li	0.
37	Pr pr*	Coup.	7 Pr- : 13 pr pr	9.60

* The marker character classified was purple vs. nonpurple grain.

When the two segregations with V v are added together a X^2 value of 9.72 is obtained. If E e and Log log are governed by the same gene as proposed by Robertson et al. (37), then these two populations may be added to give a X^2 value of 3.85 which is just significant at the 5% level of significance (1 D.F.). These results further indicate that the gene(s) conditioning the striping are on linkage group I.

The principal data in Table X were analyzed on the assumption that two genes for striping in "cornstalk" segregate to give a 13:3 ratio. However, the second set of phenotypic frequencies with the V v marker gene (population of 60 plants) is from a cross to Acc. #257 (43). Acc. #257 was mentioned previously as containing identical or allelic genes to those of "cornstalk" for striping. This small population does not give a significant deviation from either a 3:1 or a 13:3 ratio and the X^2 value was calculated on the basis of a 3:1 segregation. (For an explanation of the last column of Table X, see the Discussion).

Discussion

If we assume that two genes exhibiting dominant plus recessive epistatic interaction govern the inheritance of the striping we obtain a 13:3 theoretical ratio. The results of the F_2 data from the cross of the "cornstalk" line to Nigrinudum gave a close fit to this ratio.

	Observed	Theoretical	
13	479	480.1875	$X^2 = 0.016$
3	112	110.8125	

The dominance relations of one of the genes appear to be quite different in the crosses with Brachytic from that in the cross with Nigrinudum. If we assume that the epistasis of both genes in the cross with Brachytic lines is dominant, we obtain a theoretical ratio of 15 green to 1 striped. Combining the F_2 values for the crosses with

Table X. F_2 segregations of character pairs from the cross of Acc. #8 with the striping of the "cornstalk" mutant, Acc. #263 (13:3 ratio)

Linkage group	Phase of cross	Genotypes tested		F_2 phenotypic frequencies				Total	(13:3)	(3:1)	(X^2_L) Level of** probability	(3:1)(3:1)
		X x	Y y	X Y	X y	x Y	x Y		X^2_T	X^2_L		X^2_L
I	Coupling	V v	Cs cs*	363	73	118	39	593	5.764	5.171	.02 - .03	3.725
	Coupling***	V v	"	35	9	9	7	60			.05 - .10	3.590
	Total	V v	"	398	82	127	46	653	9.031	7.960	.01	5.825
II	Coupling	B b	"	348	83	131	28	590	1.421	0.223	.5 - .7	0.332
III-VII	Repulsion	N n	"	371	90	109	22	592	3.079	0.467	.3 - .5	0.147
V	Coupling	R r	"	384	81	97	31	593	6.421	2.728	.05 - .1	3.118
	Repulsion	S s	"	207	52	64	8	331	4.600	2.619	.1 - .2	1.598

* The symbols of Cs cs are used only for convenience to indicate the "cornstalk" striping.

** The level of probability is based on the X^2_L value for the (3:1) (13:3) ratios.

*** The parent line of the striping was Acc. #257.

Brachytic lines we obtain

	Observed	Theoretical	
15	313	318.75	$X^2 = 1.660$
1	27	21.25	

It can only be suggested that two genes giving epistatic interaction are present in these crosses. F_3 progenies would be required for conclusive proof of the tentative hypothesis.

It is also of interest to note that Arny (3) obtained results indicating that a major and a "modifier" gene for resistance to Barley Stripe were present in crosses with Brachytic.

The X^2_L values in the last column of Table X (on the probably false assumption that a single locus governs striping) has been included to satisfy the author's curiosity (and perhaps that of others) on the validity of the X^2_L values where one of the other components of the total X^2 give a significant deviation from the expected. It is gratifying to observe that where significant or large deviations for the X^2_L for the 13:3 vs. 3:1 ratios are present, these trends are also indicated in the X^2_L value for the 3:1 vs. 3:1 ratios. Therefore, even if the frequencies of the F_2 phenotypes do not fit simple ratios too well, it may still be possible to detect linkage with approximate ratios where the X^2_L may be easily calculated.

In view of the high X^2_L values found between the marker V v and the striping, it appears that at least one of the genes for the

striping is associated with linkage group I. (Further studies with linkage group V markers might also be rewarding). Using the tables of Allard (1), a recombination value of 40.92 ± 3.20 is obtained for the striping to V v on the basis of (3:1) (13:3) ratios.

Although the populations of the striped plants from the F_2 populations grown in the summer of 1958 are not large enough to calculate linkage values, the author is of the opinion that the segregations confirm the association of striping with linkage group I. In the planning of further linkage studies, the data would suggest that the striping gene is to the right of V v.

In conclusion, further investigations are required to elucidate the inheritance of the "cornstalk" mutant. In F_2 populations, the reduced tillering and the large stems cannot be distinguished on normal green plants and very few of the striped F_2 plants show indications of this expression. It appears that the reduction of tillering and the large stems are related to the amount of striping that occurs. If the striping appears early on the plants and shows severe chlorophyll deficiency, then the few large tillers appear. In most F_2 plants the striping shows up later, after tillering has been well started, and thus the reduction of tillering and the large thick stems are not apparent. Therefore, non-striped F_2 plants would not exhibit reduced tillering, which agrees with the actual observations. It is suggested that the reduced tillering and the large stems are a physiological effect of the striping, which in turn is most likely the physiological effect of a gene mutation.

According to the rating system (7), "cornstalk" should be "8" (a relatively unusable marker character due to its poor expression or complex inheritance).

2. The association of two genes, "yellow stripe" and "absent lower laterals" with linkage group VI

The mutant "yellow stripe" (Acc. #326, Table III), is one of the rarer viable types of chlorophyll-deficient mutants (39) found in barley. The distinguishing characteristic of this line is the presence of longitudinal yellow stripes (Fig. 3) which persist on the leaves from the seedling to the mature plant stage. The symbol ys is proposed for "yellow stripe", based on the similarity of this mutant to Beadle's (1929) description of the "yellow stripe" (ys_1) mutant in maize (44). The extent of the striping is quite variable in the parental and the F_2 plants, as may be expected with most chlorophyll-deficient mutants (39).

The second mutant, "absent lower laterals" (Acc. #281, Table III), is shown in Fig. 4. Fig. 4a demonstrates the distinctive growth habit of the mutant line while Fig. 4b illustrates the absence of side spikelets at the base of the head (the feature used for classification in this study). The tillers are unusually large, coarse, very stiff, and few in number, the total being only 1 to 2 (and occasionally 3) per plant, although 4 or 5 are occasionally observed in F_2 populations.

(a)

(b)

Fig. 3. "yellow stripe" (ys) - illustrating the variation in extent of striping in a homozygous ys ys line.

(a)

(b)

Fig. 4. "absent lower lateral" (als).
(a) Growth habit. (b) Side florets absent at the base of heads.

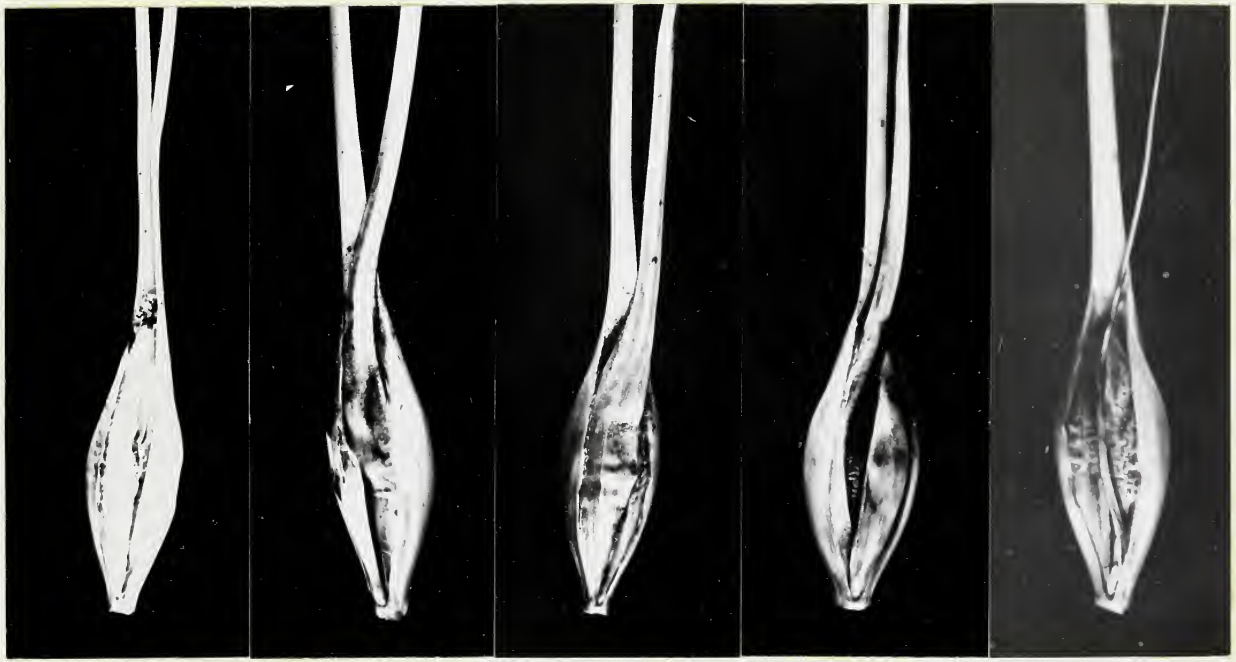
Other head abnormalities (Fig. 5) occasionally associated with the mutant consist of a slight amount of sterility (5 - 10%), awns arising from the rachilla position or the tip of the palea, and indications of triple-awned lemma.

All of the features of the parental plants of Acc. #281, and particularly growth habit, were very similar to those of the mutant "uniculm" (34).

Results

To check on the viability of "yellow stripe", F_2 populations were grown in the summer of 1957 from preliminary crosses with Acc. Nos. 8 and 11. The proportion of striped plants which survived averaged only 80% as some of the most heavily striped F_2 plants either did not reach maturity or did not survive beyond the seedling stage. From F_2's of crosses with chromosomal interchanges grown in the summer of 1958, 267 from a total of 1086 plants were ys ys, which gave a X^2 value of 0.099 for the deviation from the expected 3:1 ratio of a single recessive gene. Von Wettstein (45) stated that only rarely will translocations exhibit a negative effect on the vigour of a plant. Thus it appears that the reduction in viability from the crosses with Acc. Nos. 8 and 11 was the result of recombination with other genes from the marker lines which have slightly deleterious effects on plant vigour.

To check for the possibility of cytoplasmic inheritance of ys ys, reciprocal crosses were made with the parental variety Gateway. The F_1 plants from both crosses were normal green while the F_2 populations segregated in a ratio of 3 green to 1 striped, indicating that striping is not cytoplasmically inherited.



(a) (b) (c) (d) (e)

Fig. 5. "absent lower lateral" - illustration of awn and rachilla abnormalities. (a) indications of triple-awned lemma. (b) and (c) awned palea. (d) awn arising from rachilla position. (e) elongated rachilla.

Although the uniculm mutant appeared similar to "absent lower laterals" in all respects, the F_2 populations grown in the summer of 1958 were checked for a possible segregation of the growth habit and absent side spikelets at the base of the head. In one population, resulting from a cross with Acc. #388 (Rb rb), three plants were observed which expressed the reduced tillering (3 to 4 tillers) and the "absent lower laterals." These plants were distinct from normal plants; however, the large coarse stem was not too apparent. In the F_2 populations (several hundred plants) classified for "absent lower laterals," these mutant plants could easily be observed by their association with the uniculm-like growth habit. The cross of "absent lower laterals" for which the symbol als is proposed, to uniculm has not been made.

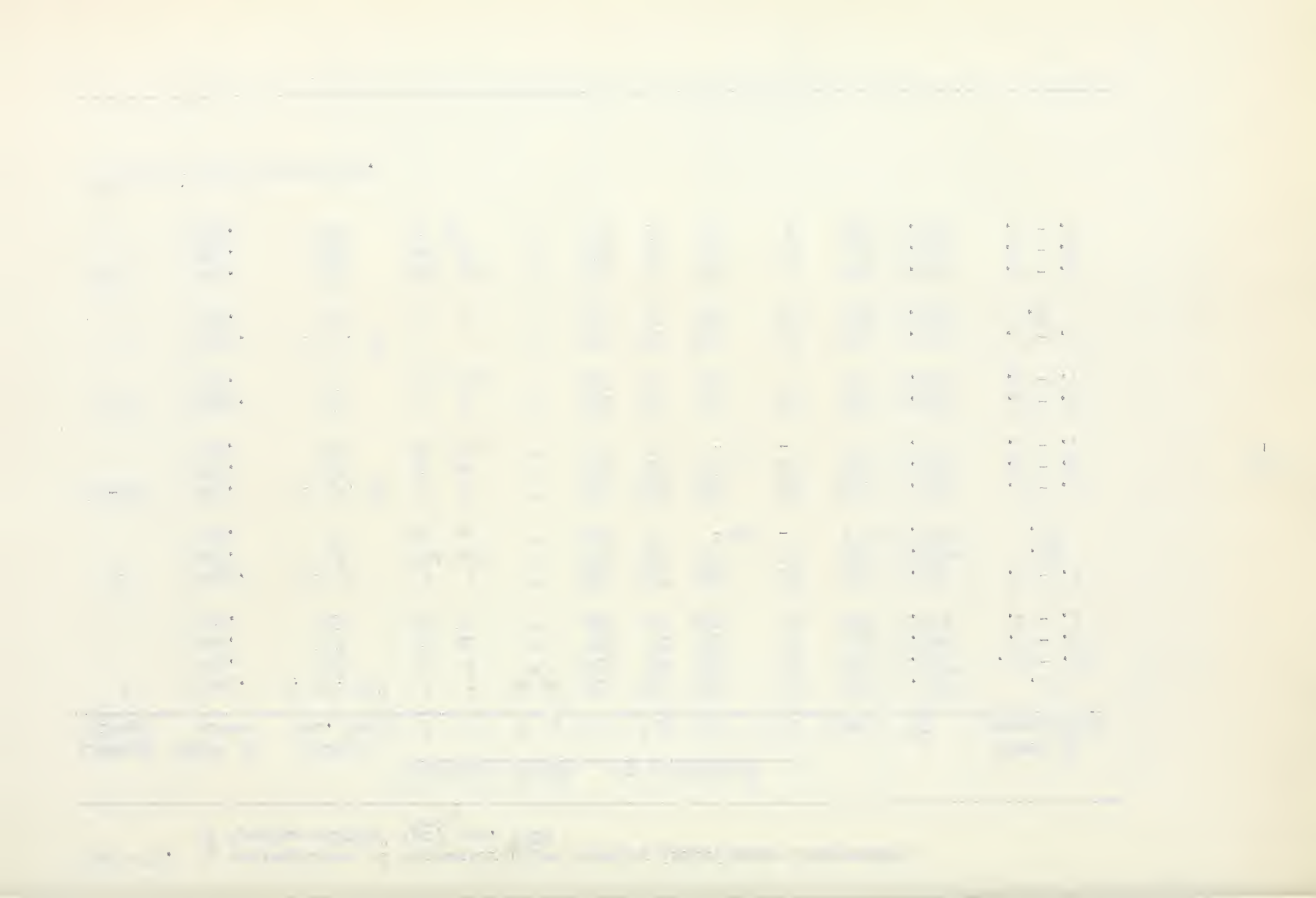
F_1 plants of crosses with Acc. #281 as the female parent, were normal for head and plant type and completely fertile. Of the numerous F_2 populations that were grown (pages 46 and 48), only the one segregating for the Trd trd marker gene gave a significant deviation (X^2 = 14.65) from a 3:1 expected ratio of Als- to als als. This deviation was likely the result of classification discrepancies. The results, therefore, showed that the mutant (Acc. #281) was conditioned by a single recessive gene.

Table XI has been prepared to show the interaction of ys ys with various marker genes in F_2 populations. The results indicate the segregation of Ys ys to be independent of marker genes in linkage groups I, II, III-VII, IV and V as well as the unlocated genes for

Table XI. F_2 segregations of character pairs showing inheritance independent of "yellow stripe" (ys) Acc. #326

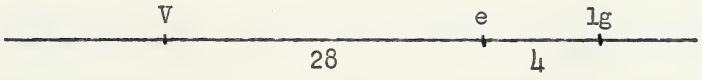
Linkage group	Phase of cross	Marker Acc. No(s)	Genotypes tested X	x	Y	y	F_2 phenotypes X Y	X y	x Y	x y	Total	X^2_L	Level of probability
I	Coup.	8, 82, 147	V	v	Ys	ys	821	199	240	80	1340	4.160*	.04
	Rep.	147	E	e	"		214	68	101	18	401	4.755*	.03 - .04
	Rep.	147	Tr	tr	"		270	81	45	5	401	1.100	.2 - .3
	Rep.	123	Lg	lg	"		325	96	113	24	558	1.473	.2 - .3
II	Coup.	8, 82	B	b	"		551	137	196	55	939	0.261	.5 - .7
	Rep.	13	Trd	trd	"		209	49	78	19	335	0.000+	.98
	Rep.	5	A_t	a_t	"		198	32	- 48	-	278	15.077*	.01
III-VII	Rep.	8, 11, 82	N	n	"		859	223	274	81	1473	0.728	.3 - .5
	Rep.	11	Br	br	"		296	92	91	19	498	1.500	.2 - .3
	Rep.	376	Yc	yc	"		50	19	- 22	-	91	0.237	.5 - .7
IV	Coup.	82	K	k	"		165	40	62	20	287	0.589	.3 - .5
	Rep.	371	Gl_2	gl_2	"		150	35	45	7	237	0.342	.5 - .7
V	Coup.	8, 11, 82	R	r	"		722	213	246	63	1244	0.694	.3 - .5
	Rep.	11	S	s	"		293	77	94	34	498	1.650	.2
Not	Rep.	359	Fs_2	fs_2	"		187	36	45	5	273	0.049	.8 - .9
	Rep.	388	Rb	rb	"		276	59	65	13	413	0.119	.7 - .8
	Rep.	259	Gp	gp	"		55	30	16	4	105	2.338	.1 - .2

* See text for explanation.



normal vs. ribbon grass (Rb rb)*, normal vs. grandpa (Gp gp)**, and normal vs. fragile stem (Fs_2 fs_2)***.

The X^2_L value for linkage of ys ys to E e on linkage group I was just significant at the 5% level (Table XI). However, the V v marker gene was also segregating in this same population and gave a X^2_L value of 1.475 with a p value of 0.2 to 0.3 indicating it was not linked to Ys ys. The gene order for linkage group I is as shown in the following diagram (37) with the accepted distances between loci.



This cross indicates a crossover value of 41.87 ± 3.40% between Ys ys and E e and no linkage between Ys ys and V v, while the cross of Lg lg to Ys ys (Table XI) indicates Lg lg is inherited independently of Ys ys. Therefore, linkage between Ys ys and E e is doubtful and the deviations shown by the X^2_L value are not caused by linkage.

Similarly the crossover value of the V v segregation of which the X^2_L just becomes significant when the data from three crosses are combined, can not be reconciled with the existing linkage data. Therefore, the

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* Woodward (46) found linkage associations for Rb rb with three different markers in linkage group IV but due to discrepancies in the values obtained, did not definitely conclude linkage associations were present.

** Burnham et al. (8) showed linkage of Gp gp with a b-g translocation. Therefore this gene is either on linkage group III-VII or the new group VII.

*** Takahashi (personal communication) indicated that Fs_2 fs_2 was associated with linkage group II.

"yellow stripe" gene is not likely associated with linkage group I. These deviations of the X^2_L for V v and E e may result from the failure (as mentioned earlier) of some of the striped F_2 plants to reach maturity.

The significant X^2 value (Table XI) for the F_2 data segregating for the A_t a_t marker gene on linkage group II results from the deviation of Ys- vs. ys ys from the expected 3:1 ratio. Linkage is obviously not involved as the deviation of the X Y and X y phenotypic frequencies occurs in the opposite direction to that expected for linkage data in the repulsion phase.

Table XII shows the F_2 interrelations of als with various marker genes, indicating that Als als segregations are independent of the markers on linkage groups I, II, III-VII, IV and V, as well as the unlocated genes rb and gp. In linkage group I, the X^2_L value is highly significant, indicating that als is not independent of Tr tr. Two possible explanations may be given:

1. That the als als locus is on group I.
2. A second gene for triple awned lemma may exist in the mutant line Acc. #281 (als).

In all the other F_2 populations segregating for Tr tr, the expression of the recessive tr was very weak (only the occasional floret per spike exhibited the triple awns), resulting in a lowered percentage of tr tr plants and giving a significant deviation from the expected 3:1 ratio of Tr- : tr tr F_2 phenotypes. The largest deviation was in the F_2 population resulting from a cross with Ys ys where only 12.47% of 401 plants were observed to express the triple awned lemma recessive character, giving a X^2 value of 33.584 (very highly significant).

Table XII. F_2 segregations of character pairs showing inheritance independent of "absent lower laterals" (als) Acc. #281

Linkage group	Phase of cross	Marker Acc. No(s)	Genotypes tested X x	Y y	F_2 phenotypic frequencies X Y	X y	x Y	x y	Total	X^2_L	Level of probability
I	Coup.	8, 147	V v	Als als	339	108	127	43	617	0.079	.7 - .8
	Rep.	147	E e	"	226	74	67	18	385	0.345	.5 - .7
	Rep.	147	Tr tr	"	218	37	75	55	385	41.018*	<.01
	Rep.	123	Lg lg	"	191	58	59	17	325	0.017	.9
II	Coup.	8	B b	"	128	44	45	15	232	0.008	.95 - .98
	Rep.	13	Trd trd	"	219	45	88	15	367	0.613	.3 - .5
III-VII	Rep.	8, 11	N n	"	170	59	54	20	303	0.044	.8 - .9
	Rep.	11	Br br	"	320	129	104	29	582	2.658	.1
	Rep.	376	Yc yc	"	105	32	- 52	-	189	0.197	.5 - .7
	Rep.	9	Fc fc	"	232	72	68	18	390	0.082	.7 - .8
IV	Coup.	9	K k	"	227	74	73	16	390	1.396	.2 - .3
	Rep.	371	Gl_2 gl_2	"	210	56	48	11	325	0.003	.95
V	Coup.	8, 11	R r	"	323	122	102	35	582	0.221	.5 - .7
	Rep.	8	S s	"	64	10	17	4	95	0.422	.5 - .7
Not located	Rep.	388	Rb rb	"	216	66	62	20	364	0.044	.8 - .9
	Rep.	259	Gp gp	"	144	39	61	15	259	0.388	.5 - .7

* See text for explanation.

The observation of a poor penetrance of tr tr in the parent line Acc. #281 combined with a 23.90% penetrance of tr tr in this F_2 population, grown under similar conditions in the same field and in the same season as other F_2 populations showing poor tr penetrance, indicates that at least two tr genes are present. The cross with the Tr tr marker is in repulsion whereas the large tr tr als als phenotypic group indicates linkage in the coupling phase. Therefore a second gene for triple awned lemma, tr_2, appears to be present in the mutant line Acc. #281, closely linked with the als locus.

An observation check for the expression of tr_2 was made on F_2 populations of Acc. #281 crossed with Acc. Nos 388 (rb) and 259 (gp). None of the normal Als phenotypes expressed the recessive tr_2 character and only a small number (approximately $\frac{1}{4}$) of the als phenotypes showed any expression of tr_2.

In conclusion, it appears that two independently inherited tr genes are present and that due to the weak expression of the tr character, the observed penetrance is low. If one of these genes is closely or completely linked with the als locus, an F_2 population ratio similar to the one segregating for Tr tr in Table XII could be obtained.

Table XIII shows the various F_2 interrelations of Ys ys and Als als with marker genes in linkage group VI. The results indicate that Ys ys and Als als are located in this linkage group.

Table XIV shows the F_2 interrelations of Ys ys and Als als with breakage points of the five marker translocations which are listed in Table V. This datum shows linkage relations of both the

Table XIII. F_2 linkage associations of Ys ys and Als als with marker gene pairs on linkage group VI

Phase of cross	Marker Acc. #	Genotypes tested				F_2 phenotypic frequencies							F_2 recombination value
		X	x	Y	y	X y	X y	x Y		x y	Total	X^2_L	
Repulsion	375	Uz	uz	Als	als	150	66	67		8	291	11.962	31.2 ± 5.2%
"	386	St	st	"		177	80	87		0	344	33.907	---
"	8	An	an	"		201	89	-	85	-	375	4.842*	28.0 ± 14.7%
Repulsion	375	Uz	uz	Ys	ys	185	62	87		1	335	21.230	---
"	359	"		"		163	41	59		0	273	7.639	---
Total	375 + 359	Uz	uz	Ys	ys	348	103	146		1	603	27.796	11.2 ± 4.0%
Repulsion	8 (Field)**	An	an	Ys	ys	85	42	-	45	-	172	4.412*	---
	8 (Sdlg)	"		"		249	114	-	112	-	475	7.942*	---
Total		An	an	Ys	ys	334	156	-	157	-	647	12.215*	18.5 ± 17.2%
Repulsion	281	Als	als	Ys	ys	95	48	35		3	181	9.901	26.3 ± 6.8%

* The X^2 value used in these instances are based on the fit to a 3:1 ratio of the X Y and X y phenotypes, since only these two phenotypes supply information for the calculation of linkage from a 9:3:4 F_2 segregation.

** From the field population, the two viable phenotypes were harvested for F_3 progenies. Seedling populations were grown in foil cake plates for classification purposes only.

Table XIV. F_2 segregations with translocations, showing linkage associations of Ys ys and Als als to the c chromosome of barley

Translocation	Mutant	F_2 phenotypic frequencies							
	Y y	S Y*	F Y*	S y	F y	Total	X^2_L**	Level of probability	Recombination value
C 1358 (b-d)	Ys ys	89	62	21	24	196	2.204	.1 - .2	
C 1483 (b-g)	"	79	64	27	21	191	0.016	.9	
C 1405 (c-d)	"	140	71	2	54	267	63.202	very small	1.75 ± 1.12
C 1432 (c-e)	"	82	37	2	48	169	66.053	very small	1.75 ± 1.40
C 1420 (e-f)	"	105	90	37	31	263	0.011	.9 - .95	
C 1358 (b-d)	Als als	86	73	29	15	203	1.381	.2 - .3	
C 1483 (b-g)	Als als	96	79	32	20	227	0.530	.3 - .5	
C 1405 (c-d)	"	120	84	25	38	267	7.022	< .01	23.33 ± 5.12
C1432 (c-e)	"	92	55	17	26	190	7.186	< .01	20.79 ± 5.12
C 1420 (e-f)	"	101	58	29	20	208	0.410	.5 - .7	

* S and F represent semi-sterile and fertile plants respectively.

** There is a deviation of S to F from the expected 1:1 phenotypic ratio present in all F_2 populations. This deviation is taken into account in the X^2_L value (28).

Ys ys and the Als als genes with translocations involving chromosomes c-e and c-d. It is therefore concluded that the c chromosome, which contains linkage group VI (Table II), carries the Ys ys and Als als genes.

These translocation crosses have yielded the following additional information; that the translocation points of C 1405 and C 1432 are very close together as well as very close to the Ys ys locus; and therefore may give an approximate distance between the Ys ys and Als als loci if the interstitial region* is not in that region of the chromosome. To explain, the distances C 1405 to ys = 1.75 and C 1405 to als = 23.33, so the distance ys to als is 21.58 to 25.08. Similarly, with the translocation C 1432, the distance ys to als is 19.04 to 22.54. Therefore the value where these ranges overlap is probably the closest to being correct, so that the distance is approximately 22 crossover units.

F_3 progenies, classifiable in the seedling stage, from crosses with marker genes were grown in the greenhouse in August, 1958, to increase the accuracy of F_2 recombination values. Progenies of the following F_2 phenotypes were selected:

(a) A_n- Ys- and A_n- ys ys (doubly and singly dominant F_2's)

(b) Uz- ys ys (singly dominant F_2's)

(c) A_n- als als (singly dominant F_2's)

(d) St- als als (singly dominant F_2's).

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* The interstitial region is that part of the chromosome between the centromere and the translocation point, in which crossing-over is said to be reduced due to the decrease of viable crossover gametes.

All of these F_3 lines germinated very well so that no less than 16 plants were observed in each line, allowing F_2 genotypic classification at the 99% confidence level.

The doubly dominant $\underline{A_n}$- $\underline{Ys}$ F_2's, when grown in F_3 lines, consisted of 0 $\underline{A_n}$ $\underline{A_n}$ $\underline{Ys}$ $\underline{Ys}$ to 10 $\underline{A_n}$ $\underline{A_n}$ $\underline{Ys}$ $\underline{ys}$ to 6 $\underline{A_n}$ $\underline{a_n}$ $\underline{Ys}$ $\underline{Ys}$ to 68 $\underline{A_n}$ $\underline{a_n}$ $\underline{Ys}$ $\underline{ys}$ genotypes as shown in the following diagram.

	A_n A_n	A_n a_n
Ys Ys	0	10
Ys ys	6	68
ys ys	33	9

Using the formulae and tables of Allard (1) a recombination value of 10.07 ± 2.50% was calculated.

The corresponding linkage value obtained from the singly dominant $\underline{A_n}$- $\underline{ys}$ $\underline{ys}$ F_2 phenotypes, which consisted of 33 $\underline{A_n}$ $\underline{A_n}$ to 9 $\underline{A_n}$ $\underline{a_n}$, was 12.00 ± 3.38%. The linkage values for singly dominant F_2 genotypes were calculated from Table 3 of Immer and Henderson (21).

The F_3 data from (c) gave 43 $\underline{A_n}$ $\underline{A_n}$ to 45 $\underline{A_n}$ $\underline{a_n}$ genotypes and a recombination value of 34.35 ± 4.78%.

The F_3 lines from (d) did not segregate for streaked, so that the recombination value appears to be zero or very close to zero.

The F_3 lines from (b) indicated the F_2 genotype consisted of 121 $\underline{Ys}$ $\underline{Ys}$ to 9 $\underline{Ys}$ $\underline{ys}$, giving a recombination value of 3.59 ± 1.19%.

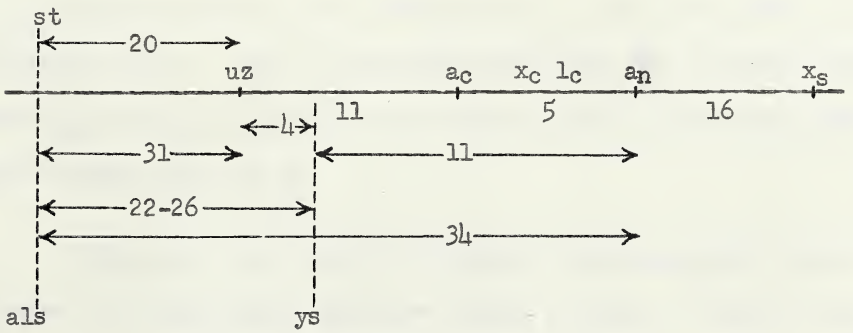
The linkage values resulting from the F_2 and F_3 data were weighted according to the amount of information supplied by each value (Kramer and Burnham, 25) and then combined to obtain best fit values as given in Table XV.

Table XV. The average weighted recombination percentages with their standard errors obtained from combining F_2 and F_3 data for the genes ys and als

Character combination	Percentage recombination
Yellow stripe and albino seedlings (Ys ys - A_n a_n)	10.84 ± 2.00%
Yellow stripe and uzu (Ys ys - Uz uz)	4.16 ± 1.14%
Absent lower laterals and albino seedlings (Als als - A_n a_n)	33.74 ± 4.57%
Absent lower laterals and streaked (Als als - St st)	zero (or very close)

Discussion

Table I (adapted from Table 3 of Burnham and Hagberg, 7) shows the following map for linkage group VI, with the accepted distance between certain loci given directly below the line.



The proposed positions of the genes located in this study are given by dotted lines perpendicular to the line of the chromosome, and the distances obtained with certain marker genes are drawn in with lines and arrows.

The distance between uz - a_n = 16 crossover units (7, 37) while the values from Table XV, uz - ys = 4.16 and ys - a_n = 10.84, give a reasonable value of uz - a_n = 15 crossover units when the Ys ys locus is between these two marker genes.

The als gene position is not as clear. The value of als - a_n = 33.74 and the F_2 value als - ys = 26.3 (probably slightly high, in view of the translocation data - see page 49) when combined, place als to the left of uz. The F_2 value for als - uz (31.0) does not agree too well with these data. However, unpublished data from other workers indicate that uz - st = 20 which with the approximately zero value obtained between als and st, fairly well substantiates the conclusion that als is to the left of uz.

Dr. Walker and associates found no recombinants between Uz uz and St st in an F_2 population of 296 plants. F_3 progenies are, however, necessary to obtain an accurate value since a single recombinant in this F_2 population would give a recombination value of as much as 11 to 12 crossover units. This F_2 population indicates the estimated distance between als and uz (31.0) may be too high in view of the very close linkage between als and st.

The author has found no references indicating that the inheritance of a chlorophyll-deficient mutant similar to "yellow stripe"

has been previously studied in barley. This may be because the vast majority of chlorophyll-deficient mutants could not be studied satisfactorily as they were lethal in the homozygous state. Gustafsson (13) estimated that only one in twenty-five chlorophyll mutants are viable.

The author considers that the absent lower side spikelets and the uniculm-like growth habit are completely associated. Since the growth habit may be influenced to a greater extent by environment than the head character, the symbol als is used for the mutant line.

From a study of the general features of the mutants als als and ys ys, the following rating values are proposed. The "yellow stripe" mutant should be placed in group"3"(seedling character, not lethal but of reduced viability). The "absent lower laterals" mutant is definitely normal in vigor but has a reduced number of tillers. It would best fit group"5"(adult plant characters associated with normal vigor).

3. Linkage studies with "mottled-3" (mt3)*

"mottled-3" (Acc. #280) was observed in the progeny of Montcalm irradiated with radium beryllium and it is inherited as a simple recessive. The character expression of the mutant (Fig. 6) appears in the early seedlings as definite white contracted bands across the leaves, which bend and break-over at the bands. As growth of the plant progresses, the extent of the expression on the newer

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* The symbol mt for mottled was suggested by Dr. D. W. Robertson and his associates.

(a)

(b)

(c)

Fig. 6. "mottled-3" ($\underline{mt}_3$). (a) Mottling in later stages of growth.
(b) F_3 progeny of $\underline{Fc}$ $\underline{fc}$ $\underline{mt}_3$ $\underline{mt}_3$ genotype.
(c) $\underline{fc}$ $\underline{fc}$ suppression of $\underline{mt}_3$ $\underline{mt}_3$.

leaves decreases until at heading time there may be only an occasional white blotch or spot on the latest-formed leaves and sometimes the stem. It has been observed that under warmer temperatures of germination (70° F.) the expression of the mottling is less pronounced.

Results

"mottled-3" plants in the F_2 populations were often reduced in vigor, 5 - 7 days later than normal green plants and occasionally failed to reach maturity. This lack of viability was not enough to cause a significant deviation from the expected 3:1 ratio of green to mottled plants where mature plant characters were classified.

Table XVI shows the F_2 interrelations of mt_3 with various marker genes from six linkage groups. The results indicate that the segregation of Mt_3 mt_3 is independent of: V v, E e, Tr tr, Lg lg, B b, Trd trd, N n, Br br, Fc fc, K k, Gl_2 gl_2, R r, S s, St st, and Uz uz. It also appears to be independent of Yc yc, although the F_2 population is too small to exclude the possibility of linkage entirely.

In the summer of 1957, the F_2 population involving the gene pair for chlorina (Fc fc) and Mt_3 mt_3 was very difficult to classify for the doubly homozygous recessive. From 227 plants, a classification of:

137 Mt_3- Fc- : 40 mt_3 mt_3 Fc- : 47 Mt_3- fc fc : 3 mt_3 mt_3 fc fc

was made, but was not felt to be accurate. Observations on F_3 progenies planted in the spring of 1958 indicated that fc fc suppressed the expression of mt_3 mt_3.

Table XVI. F_2 segregations of character pairs showing inheritance independent of "mottled-3" ($\underline{mt_3}$), Acc. #280

Linkage group	Phase of cross	Marker Acc. No(s)	Genotypes tested				F_2 phenotypic frequencies				Total	X^2_L	Level of probability
			X	x	Y	y	X Y	X y	x Y	x y			
I	Coup.	8, 82, 147	V	v	Mt_3	mt_3	411	123	134	33	701	0.629	.3 - .5
	Rep.	147	E	e	"		194	51	72	25	342	0.812	.3 - .5
	Rep.	147	Tr	tr	"		217	58	49	18	342	1.093	.3
	Rep.	123	Lg	lg	"		214	52	73	21	360	0.242	.5 - .7
II	Coup.	8, 82	B	b	"		216	60	63	21	360	0.400	.5 - .6
	Rep.	13	Trd	trd	"		192	73	79	31	375	0.067	.8
III-VII	Rep.	8, 11	N	n	"		425	118	122	46	711	2.213	.1 - .2
	Rep.	11	Br	br	"		206	57	61	28	352	3.414	.05 - .1
	Rep.*	9	Fc	fc	"		137	36	43	10	226	0.147	.7
	Rep.	376	Yc	yc	"		36	10	- 19	-	65	0.261	.5 - .7
IV	Coup.	9	K	k	"		138	32	52	14	236	0.068	.8
	Rep.	371	Gl_2	gl_2	"		275	80	75	25	455	0.299	.5 - .7
V	Coup.	8, 11	R	r	"		378	122	146	38	684	1.146	.2 - .3
	Rep.	8, 9, 82	S	s	"		207	60	54	13	334	0.108	.7 - .8
VI	Rep.	386	St	st	"		383	116	125	32	656	0.458	.5
	Rep.	375	Uz	uz	"		238	57	65	24	384	2.241	.1 - .2

* Corrected F_2 ratio (based on F_3 progeny check which indicated $\underline{fc}$ $\underline{fc}$ genotype suppressed the "mottled-3" expression).

Fig. 6b shows F_3 row progenies, segregating for normal green vs. chlorina (Fc fc) and homozygous for the recessive mt_3 mt_3. The fc fc plants are easily classified in the seedling stage, 2 - 3 weeks after emergence, by the pronounced yellow color of the newest formed leaves. None of these show any obvious signs of mottling (Fig. 6b). However, on close examination, occasional white spots may be observed on the leaves as shown in Fig. 6c. The Fc- mt_3 mt_3 seedlings, in contrast, show the severe banding and leaf breakage characteristic of the mt_3 mt_3 parent line.

F_3 progenies of "non-chlorina, mottled" F_2 plants segregating for chlorina confirm this masking effect of fc fc over mt_3 mt_3. The following ratios which were obtained are representative:

non-chlorina mottled	chlorina
19	3
15	5
20	7
16	6

It is obvious from the F_2 parental phenotypes (Fc- mt_3 mt_3) and the preponderance of mottled F_3's that these F_3's were all homozygous for mottled, and the fact that mottling was not apparent in the chlorinas indicates suppression.

F_3 progenies of the 3 F_2 plants originally designated as doubly homozygous recessive, as well as 7 other F_3 progenies, appeared to be homozygous for the greatly reduced white spotting, designated as the suppression effect, as well as homozygous for chlorina. None of

the F_3 progenies of F_2 plants homozygous for Fc fc exhibited seedlings with the severe mottled effect. Some of these lines, however, showed a few plants with the greatly reduced white spotting, indicating that the progenies were segregating for "mottled-3".

On the basis of suppression, the F_3 classification for this repulsion cross is:

	Fc Fc	Fc fc	fc fc
Mt_3 Mt_3	10	26	17
Mt_3 mt_3	33	65	22
mt_3 mt_3	13	23	10

giving a corrected F_2 ratio as indicated in Table XVI. From the X^2_L value of this F_2 data, it does appear that linkage is present. However, F_3 recombination values of 46.9 and 39.3 from the two singly dominant F_2 classes and 40.0 from the doubly dominant F_2 class were obtained which gave a combined value of 41.3 $\pm$ .6% recombination. It is doubtful that mt_3 is linked with group III - VII since the masking effect of the chlorina possibly causes a slight classification error. The X^2_L value obtained for Br br (which is accepted as 10 crossover units from Fc fc) is caused by a deviation in the opposite direction to that expected for linkage in repulsion phase data.

In the greenhouse, fall of 1957, mt_3 mt_3 was crossed with the two Mars translocation lines. The F_2 populations were grown in the summer of 1958 with the following phenotypic segregations:

C 1432 (c-e) 137 Mt_3 S : 102 Mt_3 F : 44 mt_3 S : 33 mt_3 F

C 1420 (e-f) 139 Mt_3 S : 103 Mt_3 F : 35 mt_3 S : 26 mt_3 F

The X^2_L value of 0.004 and 0.089 respectively lead to the conclusion that no linkages were present.

Discussion

"mottled-3" is quite different in appearance from mottled-1 and mottled-2, studied by Walker et al. (43). These appear as yellow zones and patches on only the seedling leaves and are much less pronounced, thus having a shorter classification period. "mottled-3" appears similar in expression to zebra (zb) found in barley (Burnham and Hagberg, 7) and the zb mutants described in maize (Weijer, 44). However, the white bands of the zebra in barley are less pronounced and can be observed only during the seedling stage. The F_1 seedlings of the cross mt$_3$ x zb were normal green, indicating that the mutant genes are at different loci.

Further crosses have been made to the translocation lines C 1376-2 (b-g), C 1462 (b-g), C 1483 (b-g), C 1358 (b-d), C 1405 (c-d) and Olli C 966 (d-g). "mottled-3" has also been crossed to grandpa (gp) which is either on linkage group III - VII or on the new group VII. From the results of Table XVI, a check for linkage to the new linkage group VII is the next logical procedure. For this purpose, the crosses with the chromosomal interchanges involving the g chromosome should be adequate.

P.M.C. analysis of F_1 plants has shown no visible chromosome abnormalities at metaphase.

By the system of Burnham and Hagberg (7), mt$_3$ would be given a "3" rating (seedling character, not lethal but of reduced viability) for its consideration as a marker gene.

4. Inheritance studies of "long weak basal internode" mutants

This study was planned to investigate the inheritance of "long weak basal internode" mutants observed in the progenies of irradiated seed of the variety Montcalm. A total of five mutants were studied with regard to possible mode of inheritance and with regard to linkage.

(a) The first mutant (Acc. #289) is distinguishable from the remaining ones particularly in respect to lateness of maturity. The line begins flowering about two weeks later than Montcalm and the remaining mutants, and rather than exhibiting a definite time of maturity, plants continue vegetative growth indefinitely. The line shows partial head sterility, ranging from complete to almost no sterility. It would appear that the sterility is caused by the physiological effect of late maturity and the mechanical effects of the "long weak basal internode." The basal internodes fold over immediately upon emergence of the head from the flag leaf, and these internodes frequently break off and the heads dry up. Branching out of a new stem or bract often occurs at the basal internode of the head as illustrated in Fig. 7.

The two characteristics, lateness and "long weak basal internode," appear to be pleiotropic effects of the same gene or of very closely linked genes.

(a) (b) (c)

Fig. 7. "long weak basal internode", Acc. #289 (lwb). (b) and (c) illustrate branching at the basal internode, while (a) is also characteristic of the expression of mutants, Acc. Nos. 291 - 293 (lwb_2).

Results

F_1 plants resulting from crosses of marker gene lines with this mutant (Acc. #289) exhibit basal internodes of normal length, but mature a week or so later than Montcalm. No intermediate expressions of the mutant were observed in normal F_2 plants from the above crosses. Even though the length of the basal internode varies in the very late F_2 plants, the mutants are still quite distinct.

The symbol lwb is proposed for this mutant with the "w" included to distinguish this very long and weak basal internode from the long basal internode (lb) character associated with linkage group V (37).

Table XVII shows the F_2 segregations of Lwb lwb in relation to those of various marker gene pairs from six linkage groups. The results indicate that the gene for "long weak basal internode" is associated with linkage group IV and is inherited independently of one or more marker gene pairs in each of the linkage groups I, II, III-VII, V and VI. Some of the lwb lwb plants matured too late to allow classification for some of the morphological and mature head characters (V v, B b, Trd trd, N n, K k, I^h i and R r). Their exclusion from the data gave a slightly significant deviation of Lwb : lwb from the expected 3:1 ratio. Allowances for these deviations are made by the application of X^2_L values for linkage detection in data of Table XVII.

The F_2 recombination values of Lwb lwb with K k and I^h i were

lwb - K = 12.82 ± 1.33%

and lwb = I^h = 30.54 ± 5.12%

Table XVII. F_2 segregations of marker character pairs with "long, weak basal internode" (lwb) Acc. #289

Linkage group	Phase of cross	Marker Acc. No(s)	Genotypes tested X x	Y y	F_2 phenotypic frequencies X Y	X y	x Y	x y	Total	X^2_L	Level of probability
I	Coup.	8, 13	V v	Lwb lwb	389	99	127	35	650	0.116	.7 - .8
II	Coup.	8	B b	"	342	96	97	13	548	2.920	.05 - .1
	Rep.	13	Trd trd	"	97	23	34	13	167	1.230	.2 - .3
III-VII	Rep.	8	N n	"	346	95	100	22	563	0.332	.5 - .7
	Rep.	9	Fc fc	"	210	54	73	19	356	0.000	very high
	Rep.	107	Br br	"	37	18	16	1	72	4.840	.02 - .05
	Rep.	376	Yc yc	"	103	34	- 34	-	171	0.002	.95
IV	Coup.	9	K k	"	534	42	43	123	742	289.003	very small
	Rep.	371	Gl_2 gl_2	"	76	37	34	0	147	24.218	< .01
	Rep.	8, 13	I i	"	157	52	89	7	305	15.012	< .01
	Rep.	43	Z z	"	90	28	- 42	-	160	0.102	.7 - .8
V	Coup.	8	R r	"	288	75	97	20	480	0.533	.3 - .5
VI	Rep.	360	Uz uz	"	54	27	14	7	102	0.039	.8 - .9
	Rep.	8	A_n a_n	"	220	56	- 98	-	374	3.265	.05 - .1

There were no recombinants between lwb and gl_2 and therefore F_3 progenies were grown. Poor germination necessitated the use of F_3 progenies of only 10 or more plants for classification of F_2 genotypes at the 95% level of accuracy, giving the following data for repulsion phase:

	Gl_2 Gl_2	Gl_2 gl_2	gl_2 gl_2
Lwb lwb	0	1	7
Lwb lwb	0	32	0
lwb lwb	12	0	0

As may be seen from this figure, none of the singly dominant F_2's (Lwb- gl_2 gl_2 or lwb lwb Gl_2-) segregated, while only one recombinant was obtained from the doubly dominant F_2 phenotypes. Thus, using the tables of Allard (1), a recombination value lwb - gl_2 = 1.5 ± 1.4 was obtained.

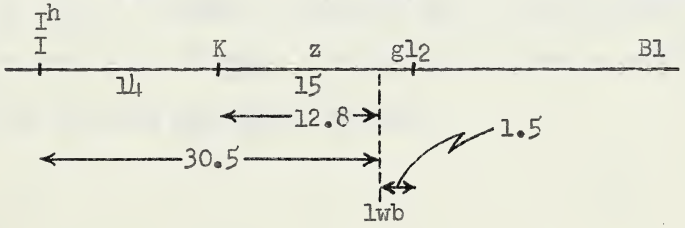
Robertson et al. (36) list the following recombination values for I^h i, K k and Gl_2 gl_2 in linkage group IV:

I^h - K = 14.3

K - gl_2 = 15.1

I^h - gl_2 = 28.0

The accepted gene order with distances are listed as follows, with the values obtained with lwb shown below the chromosome line:



The lwb gene may be on either side of gl_2, although the value for the distance to K k has a very low standard error which would indicate a preference for lwb being to the left of gl_2.

The marker gene for normal vs. zoned leaf (Z z) has been associated with linkage group IV, between K k and Gl_2 gl_2. However, the F_2 segregation of Z z with Lwb lwb did not show linkage indications (Table XVII).

The F_3 progenies, which gave a 95 percent confidence level of accuracy for the classification into F_2 genotypes of the doubly dominant F_2 phenotype, gave the following segregations in the repulsion phase:

	Z Z	Z z
Lwb Lwb	1	5
Lwb lwb	4	22

These data give a recombination value of 19.5 ± 6.1%. Robertson et al. (36) listed the following values

K - z = 6, z - gl = 3 and K - gl = 10,

from which gl - gl_2 would be about 5 crossover units apart. Recently, Robertson (34) quoted the values of

z - gl = 9.3 and 14.0 while K - z = 0, 12.8 and 14.0

from one report and K - z = 27.0 and 35.5 from another. The position of Z z on group IV cannot be definite due to these latest results and therefore the value of lwb - z = 19.5 ± 6.1 does not add further information for locating Lwb lwb at present.

Discussion

The F_1 plants of the cross of Lwb lwb with Z z were observed to be faintly zoned, very weak and not viable in all instances. In a small F_2 population of this cross, also grown under greenhouse conditions as were the F_1's, the faint zoning appeared on a large number of the seedlings. This zoning soon changed to green in a number of the plants (later shown to be heterozygous for zoned). Since the zoned marker is considered to be recessive (34), a deletion was suspected in the lwb lwb line. To test the possible area covered if the deletion were present, reciprocal crosses of lwb were made with gl, gl_2, lg_2, lg_3 and lg_4 genes, all of which are positioned close to the Z z locus. All F_1 seedlings were normal green, indicating that these loci were not in the region of the possible deletion. No further tests were possible to check the presence of a deletion.

One other explanation of the zoned results appears quite feasible. Since the parental line of zoned is not viable under greenhouse conditions but fully viable in the field, the heterozygous Z z condition may show a slight effect on seedlings grown under greenhouse conditions.

The cross with zoned appeared to be segregating for a gene, independent of lwb, causing moderate elongation of the basal internode. Other F_2 and F_3 populations showed no indications of possessing this secondary character and hence it is assumed to have been present in the zoned parent.

(b) Three other "long weak basal internode" lines (Acc. Nos. 291, 292 and 293) have an expression of the basal internode very similar to that of Acc. 289 (see first illustration of Fig. 7). A fourth line, Acc. #290, has a somewhat shorter basal internode, possibly similar to the long basal internode caused by the gene (lb) in linkage group V (37).

Being shorter, the head does not fall over very often, compared with all the other lines. These lines mature in approximately the same length of time as the parental variety Montcalm.

Results

These four lines were intercrossed and crossed with Acc. #289 (lwb) and the lb gene (Acc. #385) on linkage group V to check for possible allelism. The results are listed as follows:

Acc. Nos. crossed	F_1 observations
289 x 290	Fertile - normal
289 x 292	5-10% sterile - normal
289 x 293	Fertile - normal
290 x 291	25-50% sterile - moderately long basal internode
290 x 292	Fertile - long basal internode
290 x 293	Fertile - long basal internode
291 x 293	Fertile - long basal internode
292 x 293	Fertile - long basal internode
290 x 385	Fertile - normal
291 x 385	Fertile - normal
292 x 385	Fertile - normal
293 x 385	Fertile - normal

The results of the intercrosses indicate that the "long weak basal internode" (lwb) gene in Acc. #289 and the lb gene on group V (Acc. #385) are not alleles of the other four lines tested (Acc. Nos. 290 - 293). On the other hand, the "long weak basal internode" characters in these other lines are probably conditioned by allelic or identical mutant genes (tests for pseudoalleles were not undertaken).

A chromosomal interchange was suspected to be involved in the cross of Acc. Nos. 290 x 291; however, cytological observations of P.M.C. divisions gave no indication of a translocation being present.

F_2 progenies of the four lines, 290 to 293, were grown from F_1's of crosses with Acc. Nos. 8 and 11 made by Dr. Walker. The classification of "long weak basal internode" plants was easy in the crosses involving Acc. Nos. 291, 292 and 293, owing to the marked expression of the character, while the classification of F_2's involving Acc. #290 was difficult. For classification purposes of these latter progenies, the plants that were on the borderline between the moderately long basal internode and normal were omitted from the calculation and the X^2_L value was relied on to detect linkage.

Table XVIII shows the F_2 interrelations of "long weak basal internode" mutants (Acc. Nos. 290, 291, 292, 293) with various marker genes. The F_2 results of Acc. Nos. 291, 292, and 293 were calculated separately. However, as the data and character expressions were very similar and intercrosses indicated they were allelic, the results have been combined in Table XVIII. These results indicate that the genes concerned are linked with hulled vs. naked caryopsis (N n) in

Table XVIII.* F_2 segregations of marker character pairs with "long weak basal internode" mutants Acc. Nos. 291, 292, 293 (combined), and 290

Linkage group	Phase of cross	Marker Acc. No(s)	Genotypes tested X x	Y y	F_2 phenotypic frequencies X Y	X y	x Y	x y	Total	X^2_L	Level of probability
(a) Accession lines 291, 292 and 293 combined:											
I	Coup.	8	V v	Lwb_2 lwb_2	401	143	123	34	701	1.313	.2 - .3
II	Coup.	8	B b	"	397	132	127	45	701	0.099	.7 - .8
III-VII	Rep.	8, 11	N n	"	643	363	319	16	1341	131.335	very small
	Rep.	11	Br br	"	336	153	102	49	640	0.025	.8 - .9
V	Coup.	8, 11	R r	"	594	229	137	63	1023	0.431	.5 - .7
	Rep.	8	S s	"	405	131	119	46	701	0.755	.3 - .5
VI	Rep.	8	A_n a_n	"	92	32	- 38	-	162	0.043	.8 - .9
(b) Accession line 290:											
I	Coup.	8	V v	Lb_2 lb_2	173	14	26	29	242	45.269	very small
II	Coup.	8	B b	"	158	33	41	10	242	0.310	.5 - .7
III-VII	Rep.	8, 11	N n	"	411	120	150	22	703	6.385	.01 - .02
	Rep.	11	Br br	"	278	83	84	28	473	0.198	.5 - .7
V	Coup.	8, 11	R r	"	210	49	58	18	335	0.863	.3 - .5
	Rep.	8	S s	"	159	33	40	10	242	0.413	.5 - .7

* Includes only F_2 populations grown in the summers of 1956 and 1957.

linkage group III - VII, with a combined recombination value of 20.03 ± 2.60%. A significant X^2_L is also obtained with N n giving a recombination value of 40.34 ± 3.12% for the mutant gene of Acc. #290. This moderately long basal internode character (Acc. #290) also showed very strong linkage with non 6-rowed vs. 6-rowed (V v) in linkage Group I, giving a recombination value of 19.53 ± 2.90.

Discussion

It became evident from the earlier F_2 analysis of these "long weak basal internode" testcrosses that genes for a minor elongation of the basal internode were segregating. When the F_2's of crosses of Acc. Nos. 290 and 292 with a tester line (Goldfoil, Acc. #377) were carefully examined it was found they could be classified in three groups with respect to the basal internode: "long and weak," "moderately long and weak" and "normal" (short and strong), as follows:

Acc. #290 x 377 F_2 (Coupling)

		165 V	65 v
9	"long"	5	4
72	"moderate"	37	35
149	normal	123	26

Acc. #292 x 377 F_2 (Coupling)

		110 V	27 v
34	"long"	26	6
29	"moderate"	22	7
74	normal	62	14

The differences between the three classes is slight in the first population, and hence the departure from a 3:1 ratio for "elongated internodes" may be caused by classification error. Nevertheless, the X^2_L between "elongated" vs. "normal" internodes and V v; and the recombination value of 30.73 ± 3.72 substantiate the earlier

results (Table XVIII) although still not allowing a decision whether this is a true or a "pseudo" linkage. This linkage, moreover, appears to be related to a minor gene (present in Acc. #290) since the recombination value (30.53 ± 3.85) is not reduced when the "long, weak" class is excluded from the calculations.

The classification for "internode type" in the second population gives a good fit to a 9:3:4 ratio (X^2 = 0.570) and also to a 9:7 ratio (X^2 = 0.278). The results may therefore be explained by the presence in Acc. #292 of two independent recessive genes, one causing the "long weak basal internode" and masking, by its drastic effect, the effect of the minor gene causing "moderately long and weak basal internode." Neither of the genes indicated appears to show linkage with the Vv locus.

The F_2 population of the interline cross Acc. # 289 x Acc. #293 could not be classified for the two types of elongation. The classification yielded 85 plants with "long weak basal internodes" and 67 with "normal" internodes.

	Observed	Theoretical
lwb 37	85	87.875
lwb 27	67	64.125
Total	152	152

This data gives a X^2 for a 37:27 ratio of 0.223. The results may be interpreted as indicating that three independent characters for the elongation of the basal internode are present. A distinction between

a "moderate" elongation class and the extreme "long" elongation classes could not be definitely drawn although both types were obviously present.

The data of the various tests on these mutants may be integrated and the various genes designated as follows:

Acc. #289 - a single gene for "long weak basal internode" (lwb) on linkage group IV.

Acc. #290 - a) a gene (lb_2) for "minor elongation" of the basal internode on linkage group I

b) a gene (lwb_2) for "long weak basal internode" on linkage group III - VII, non-masking for a).

Acc. Nos 292 and 293 - a) a gene for minor elongation of the basal internode independent of that in linkage group I, and of lb on group V

b) a gene (lwb_2) for "long weak basal internode," allelic or identical to b) of Acc. #290.

Whether the genes designated "lwb_2" are identical (in the sense of having resulted from a specific mutational event and disseminated by accidental cross pollination) cannot be decided. That present in Acc. #290 seems indeed to be different in expression from those in Acc. Nos. 291, 292 and 293.

The possibility that the "minor elongation effect" may be a partial expression of the "major effect" gene in the heterozygous state

appears to be rather remote. The F_2's of the cross involving Acc. #292 give a good fit to a 9:3:4 ratio and the intercross of Acc. Nos. 289 and 293 a good fit to the 37:27 ratio, which excludes the above probability. In addition to the specific disagreement of this hypothesis, a general disparity is that the hypothesis requires approximately 50% of the population to show "moderately elongated basal internode."

5. General discussion

Confirmation of published data

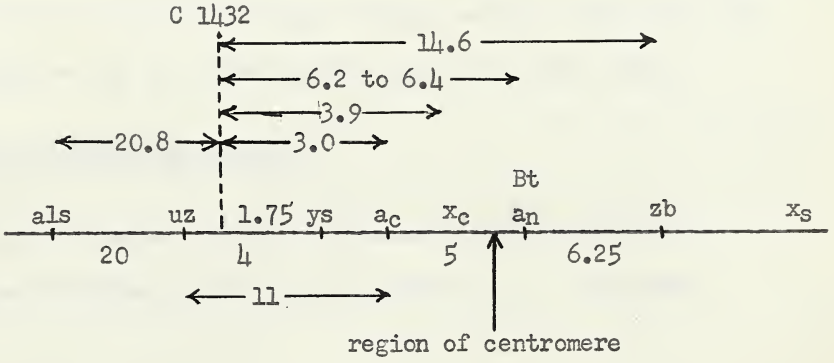
Various previously accepted linkages were checked throughout this study. In linkage group I, the relations of the very similar characters for long-awned glumes, Log log and E e, were checked. Robertson et al. (37) suggested that these characters be combined under the general symbol of E e. Intercrosses have shown that the markers Acc. #147 (E e) and Acc. #152 (Log log) used in this study are conditioned by either allelic or identical genes (tests for pseudoalleles have not been completed). The discrepancies frequently found in the recombination values to V v are most likely caused by chromosomal aberrations. Woodward (46) found crossover values of 26.5, 27.0 and approximately 50 (independence) from three different crosses with E e lines. The results of this study, v - e = 33.32 ± 4.71% and v - log = 24.37 ± 3.74%, where the Log log and E e lines used have been found to be allelic suggest the possibility of chromosomal aberrations. One line designated Log_3 log_3 was observed by Dr. Walker and associates to contain a translocation (identified in Part II of this study).

The distance between the genes for rough vs. smooth awns (R r) and long vs. short-haired rachilla (S s) was calculated to be 25.66 ± 2.41 crossover units from an F_2 population of 466 in coupling and 27.97 ± 1.57 units from an F_2 population of 1200 in repulsion. These values correspond closely to the recently reported crossover values of 21.1 and 25.99 (41) and 26.5 (46).

The position of centromere and translocation point of C 1432 on linkage group VI

Numerous linkage values have been found between the translocation point of C 1432 and various marker genes associated with linkage group VI. As has been proposed by various workers (2, 16, 17), such data may be useful in positioning the centromere in the linkage group.

Burnham et al. (8) reported the gene to breakage-point values: C 1432 - a_n = 6.42, C 432 - Bt = 6.2 (these two values agree with Takahashi's (unpublished) marker gene distance Bt - a_n = 0.86), C 432 - a_c = 3.06, C 1432 - x_c = 3.90, and C 1432 - zb = 14.6. Along with the values of C 1432 - ys = 1.75 and C 1432 - als = 20.79 obtained in this study, these data suggest the following gene order. (The values below the line are the approximate linkage values between various loci, while those above the line are the values to the breakage-point of C 1432).



Because of non-viability of crossover gametes from crossing-over in the interstitial region, the apparent recombination between genes in this region is reduced (16, 24). This reduction may be observed in the above diagram for the region including C 1432, ys, a_c, x_c and a_n. The translocation point of C 1432 may possibly lie on the side of ys opposite to that shown, but the value C 1432 - als (20.8) indicates it is to the left of ys and perhaps very close to uz.

Since the reduction of crossing-over occurs in the region between the translocation point and the centromere, the relative position of the centromere may be observed from the diagram. If we subtract the value of a_n - zb = 6.25 from zb - C 1432 = 14.6 we obtain a value of 8.35. Therefore the centromere is most likely about 2 crossover units to the left of Bt and a_n as shown. The amount of reduced crossing-over in the interstitial region is not known, but from the reduced values to a_c and x_c, one would expect the centromere to be quite close to a_n or just to the left of it.

One value from linkage data does not agree with the order based on the distance to the interchange point. Burnham et al. (8) obtained a value of zb - x_c = 1.5 so that either it or the C 1432 - zb = 14.6 value can not be accepted. The values of C 1432 to a_c, x_c, a_n and Bt are very consistent in their reduced values, indicating the gene order suggested by the translocation point is likely valid.

Evaluation of mutants as markers

For use as marker genes, particularly with translocation studies, viable "seedling" mutants are in demand (5). Characters

classifiable in seedlings allow F_3 progenies to be grown for linkage data where space is a limiting factor. The author is of the opinion that "yellow stripe" and "mottled-3" are two such useful mutants. "Mottled-3" may be in the new group VII and therefore of extreme value to barley workers as a marker gene.

The "absent lower lateral" gene gives a very distinct morphological effect. It appears to extend linkage group VI considerably beyond uz and should prove very useful for this reason.

The "long weak basal internode" (lwb_2) on group III - VII is easily classified and of normal vigor and maturity. It therefore shows good possibilities as a marker for this region where good markers are scarce. The first "long weak basal internode" (lwb) is located in a region of linkage group IV where numerous good seedling markers have been located and therefore may be of little practical value. Similarly the "sterile 'brachytic'-like dwarf" is in the area of other good markers, although the associated male-sterility and seedling detectability give it specific advantages as a marker. "Cornstalk" is presently of little value due to the uncertainty of its inheritance. Such characters which are not inherited as simple Mendelian recessives are not of practical value as marker genes.

Added Note

In a recent letter (received too late to enter this manuscript), Dr. D. W. Robertson (Colorado) suggested the following symbols for the mutants of this study.

He agreed with the symbols *ys* for "yellow stripe," *mt* for "mottled-3" and *als* for "absent lower laterals." For the "sterile 'brachytic'-like dwarf" he suggested the symbol ms_3, indicating male sterility as the major character expression. For "long weak basal internodes," lb_2 was suggested for Acc. #289 and lb_3 for the Acc. Nos 291 - 293 main factor. The suggested symbol changes may be adopted for publication purposes.

PART I

SUMMARY

The study of the inheritance and linkage of six morphological and chlorophyll-deficient characters, promising as sources of marker genes, has yielded the following information:

1. The gene for "sterile 'brachytic'-like dwarf" is located on linkage group I, 0.6 crossover units from the gene conditioning light-green seedlings (lg). The striping of "cornstalk" appears as a physiological-gene mutant that is not simply inherited. Epistatic effects of genes giving ratios of 13:3 and 15:1 are suggested to control the striping which is associated with group I.

2. Genes conditioning "yellow stripe" (ys) and "absent lower laterals" (als) are located in linkage group VI in the following gene order, als - uz - ys - a_n.

3. The gene conditioning "mottled-3" (mt_3) is inherited independently of one or more marker pairs in each of six linkage groups, leaving the possibility that this gene may be in the new group VII.

4. Two genes for "long weak basal internode" have been located: lwb, 1.5 crossover units from the gene conditioning glossy seedlings (gl_2) on group IV, and lwb_2, 20 crossover units from naked caryopsis gene (n) on group III - VII. Evidence of one or more genes conditioning a minor elongation of the basal internode, one possibly associated with group I, is presented.

5. The gene order of als - uz - ys - a_c - x_c - a_n - zb and the position of the centromere between x_c - a_n are suggested for linkage group VI on the recombination values obtained between the various marker genes and the translocation point of the translocation C 1432.

Confirmation of the position of the liguleless gene (li) on linkage group I and allelism of E e and Log log marker pairs is given.

PART II: THE CYTOLOGICAL IDENTIFICATION OF CHROMOSOMAL INTERCHANGES

MATERIALS AND METHODS

The translocation lines used in this study are listed in Table XIX. The first five were found by Dr. Walker and Mr. R. Miller in material of crosses with genetic stocks in the barley collection started by Dr. Walker in 1954. The latter two lines were supplied by Dr. Kerber.

Table XIX. Sources of new translocation lines

U. of A. Acc. #	Source designation and name	Worker
67	54/1121 r 65 Long-awned outer glumes-3	Dr. N. Nybom, Sweden
60	54/1121 r 95 Early (tidig)-6	Dr. N. Nybom, Sweden
161	L50-305 Sterile + Misshaped heads	Dr. J.W. Lambert, Minnesota
176	L50-321 Chlorotic stripe, Light green	Dr. J.W. Lambert, Minnesota
271	X5305 Dense - from irrad. Montcalm	Dr. T. Lawrence, U. of A.
466	Gateway C961-6	Dr. E. Kerber, U. of A.
467	Olli 10L-10-1-4 C963	Dr. E. Larter, U. of A.

This study was planned to identify the chromosomes that had been interchanged, following the method of intercrossing these lines to a set of tester lines similar to those used by Burnham et al. (6)*.

* Upon the suggestion of Dr. Burnham (personal communication) the line C 1385 (a-b) was replaced by C 1483 (b-g) (Table XX).

For this purpose it was necessary to ensure that plants homozygous for the translocation concerned were used. Observations of random sterility in the parental lines of the above accessions grown in the summer of 1957 indicated that some might be translocation heterozygotes. Three fertile plants were therefore selected from each of the first five lines listed in Table XIX to give a good probability of one or more being a translocation homozygote. Progenies of these were sown in the greenhouse in September, 1957, along with the variety Montcalm and five tester translocation lines from the variety Mars, as listed in Table III of Part I. Selection of homozygous translocates was not considered necessary for the remaining lines because previous tests had indicated the seed used was homozygous for the translocation.

Plants in each selection and in two progenies of each of the last two lines (Table XIX) were crossed to the tester set, and also to the variety Montcalm to check whether the fertile selection was homozygous for the translocated or non-translocated chromosomes. A further check for the presence of partial sterility was made on the selections used for crossing. This ovule-sterility check for translocation heterozygotes was rendered unreliable by the appearance of sterility in all lines due to inadequate greenhouse conditions.

The F_1's of these intercrosses were planted in the greenhouse in February, 1958. Due to space conditions, only three plants from each cross were grown. Material from each plant was taken and preserved, for cytological observation, in Carnoy's 6:3:1 solution. After 24 hours at room temperature it was stored in refrigeration at 2 - 3° C. until studied by the aceto-carmine smear method.

The method of determining the chromosomes (6) concerned in a translocation relies on the types of configuration present at first metaphase of meiosis in the F_1 hybrid between "tester" and "tested" translocation homozygotes. The following comments serve to illustrate the reasoning involved:

1. If no single chromosome pair is common to the two translocations intercrossed, then two rings of four chromosomes (2⊚4) will be observed.
2. If one chromosome pair is involved in each of the two translocations, then a ring of six (⊚6) will be observed.
3. If the same pair of chromosomes is involved in both translocations, normal pairing of the seven pairs (7^{II}) will be observed most frequently, but depending on the break positions an association of four chromosomes (⊚4) may be observed.
4. As may be seen from Table XX, chromosomes b, c, d, e, f and g are involved in translocations. By comparing configurations in a group of five crosses, one can decide which of the chromosomes are translocated in the "tested" line, and by inference decide whether chromosome a is involved.

Thus, for example, in the test of Acc. #67 (Table XX), the presence of a ring of six in crosses with C 1358 and C 1420 indicate b or d and e or f are involved. The presence of two rings of four in the crosses with C 1483 and C 1432 show that neither b nor e is involved. Hence the chromosomes involved are d and f.

Table XX. Cytological meiotic configurations of F_1 plants from crosses to a tester set of interchanges as designated by Burnham et al. (6)

Lines tested	Tester interchange set - with chromosomes involved					Chromosomes interchanged
	C 1358 b-d	C 1483 b-g	C 1432 c-e	C 1420 e-f	C 1405 c-d	
Acc. #67 (P05)	⌀6	2⌀4	2⌀4	⌀6		d-f
Acc. #60 (P06)	⌀6	2⌀4	⌀6	⌀6		d-e
Acc. #161 (P14)	7^{II}	⌀6	2⌀4			b-d
Acc. #176 (P17)	⌀6	⌀6	2⌀4	2⌀4	2⌀4	a-b
Acc. #271 (P48)	⌀6	7^{II}	2⌀4	2⌀4		b-g
Acc. #466	⌀6	⌀6	2⌀4	2⌀4	2⌀4	a-b
Acc. #467	⌀6	⌀6	2⌀4	2⌀4	⌀6	d-g

With four exceptions, all the twenty-one various chromosome combinations may be identified with the first four testers of Table XX. However, to decide whether either a-b or d-g, and whether a-c or e-f are involved, requires the use of C 1405 (c-d).

RESULTS AND DISCUSSION

The results of the study are presented in Table XX (page 84).

In this study, it was necessary to use the fifth tester (C 1405) in three instances. In Acc. Nos. 176 and 466, two rings of four were observed with this tester indicating the d chromosome was not involved, so the translocations are a-b; in Acc. #467 it is d-g.

In correspondence from Dr. Hagberg (Svalof) he mentioned that he had also observed translocations in the two lines from Nybom and that one (Acc. #67) had been identified as a d-e translocation. If his observation is correct, then the cross of Acc. #67 to C 1432 should give a ring of six configuration rather than the two rings of four obtained. Three more reserve spikes of pollen mother cell material of this cross were checked and found to contain two rings of four. However, a crossing error could be involved and this cross has been repeated with a different source of C 1432 (Burnham, May, 1958). The F_1 remains to be grown to check the results.

The translocations of Acc. Nos. 67 and 60 may be quite valuable in translocation work, since the d-f and d-e combinations are scarce at present. Until Hagberg's correspondence concerning the possibility of Acc. #67 being d-e, the author had not known of any d-e translocations in existence and of only one other d-f (produced in a two-rowed Swedish variety). Hagberg (14) observed that the b chromosome was by far the most frequently translocated chromosome, while in these results translocations involving both the b and d chromosomes were high in frequency of occurrence.

SUMMARY

As a result of this study, the chromosomes involved in six barley translocations are identified as a-b, a-b, b-d, b-g, d-e and d-g. In addition, one other translocation is concluded as either d-e or d-f.

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